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(57) Abstract

A new protein named Tankyrase II is described in this disclosure. Sequences for the human Tankyrase II cDNA and the protein translation product are provided. Also provided are species homologs, muteins, related nucleic acids, peptides, and drug screening assays. Tankyrase II interacts with telomere-associated proteins, thereby affecting telomerase activity and potentially telomere length. The materials and techniques provided in this disclosure allow Tankyrase II activity to be studied in vitro and manipulated inside cells - to the potential benefit of clinical conditions associated with a defect in telomerase activity, or the replicative capacity of affected cells.

(57) Abrégé

L'invention concerne une protéine appelée Tankyrase II, ainsi que des séquences pour l'ADN complémentaire de la Tankyrase II et le produit de la traduction des protéines. La présente invention concerne également des espèces homologues, mutéines, des acides nucléiques apparentés, des peptides ainsi que des techniques de criblage de médicaments. La Tankyrase II interagit avec des protéines associées au télomère, affectant, de ce fait, l'activité de la télomérase et éventuellement la longueur du télomère. Les matières et les techniques décrites dans la présente invention permettent l'étude in vitro de l'activité de la Tankyrase II et la manipulation de cette dernière dans des cellules, afin d'améliorer les conditions cliniques associées à une anomalie de l'activité de la télomérase ou à la capacité à se répliquer des cellules atteintes.

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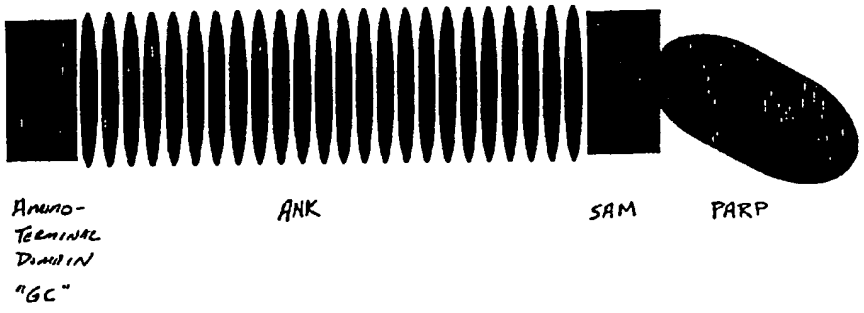

(57) Abstract

A new protein named Tankyrase II is described in this disclosure. Sequences for the human Tankyrase II cDNA and the protein translation product are provided. Also provided are species homologs, muteins, related nucleic acids, peptides, and drug screening assays. Tankyrase II interacts with telomere–associated proteins, thereby affecting telomerase activity and potentially telomere length. The materials and techniques provided in this disclosure allow Tankyrase II activity to be studied in vitro and manipulated inside cells – to the potential benefit of clinical conditions associated with a defect in telomerase activity, or the replicative capacity of affected cells.

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A SECOND MAMMALIAN TANKYRASE

RELATED APPLICATIONS

This application claims the priority basis of U.S. Provisional Patent Applications 60/128,577, filed April 9, 1999; and 60/129,123, filed April 13, 1999. For purposes of prosecution in the U.S.A., the priority documents are hereby incorporated herein by reference in their entirety.

TECHNICAL FIELD

This invention relates generally to the field of molecular biology of telomere and telomere associated proteins, and the maintenance of telomere structure. More specifically, this invention relates to a novel protein that shares three domains of homology with the telomerase associated protein Tankyrase I.

BACKGROUND

Recent research has described what may be a key switch in the control of cellular aging. The telomeres at chromosome ends are made up of multiple repeats of the DNA sequence TTAGGG, which are thought to stabilize the chromosome during replication. Telomeres shorten each time the cell divides, and cells become senescent when the telomeres are too short to protect the chromosome. But in some cells, including embryonic cells, an enzyme called telomerase rebuilds the telomeres after each division, extending the replicative capacity of the cell. (Bodnar et al., Science 279:349, 1998; Harley et al., Curr. Opin. Genet. Dev. 5:249, 1995).

Regulation of telomerase activity is a complex process involving several protein components. Two such proteins have DNA binding activity, and are named telomeric repeat binding factors (TRF1 and TRF2). It is thought that TRF1 is involved in regulating telomere length, because overexpression of wild-type TRF1 makes telomeres shorter, while overexpression of a dominant-negative form of TRF1 makes telomeres longer — perhaps by affecting the access of telomerase to the chromosome terminus (van Steensel et al., Nature, 385:740, 1997). TRF1 promotes parallel pairing of telomeric tracts, apparently pairing in parallel homodimers that form filamentous structures on longer telomeric repeat arrays (Griffith et al., J. Mol. Biol. 278:79-88, 1998).

The role of TRF2 appears to be protection of the chromosome terminus, since expression of a dominant-negative form of TRF2 leads to chromosome-chromosome fusions. (Griffith et al., J Mol Biol. 278:79, 1998; Broccoli et al., Nature Genetics 17:231, 1997; van Steensel et al., Cell 92:401, 1998). This in turn leads to p53- and ATM-dependent apoptosis of the cell (Karlseder et al., Science 283:1321, 1999). TRF1 and TRF2 have been implicated in large duplex loops at the end of telomeres that may provide a general mechanism for telomere protection and replication (Griffith et al., Cell 97:503, 1999).

Smith et al. (Science 282:1484, 1998; Genomics 57:320, 1999; J. Cell Sci. 112:3649, 1999) have reported a novel protein that associates with TRF1, which they named "Tankyrase". A yeast two-hybrid screen was used with human TRF1 as bait, and yielded two overlapping cDNAs which provided the full-length sequence. Northern blot analysis revealed that multiple mRNAs were ubiquitously expressed in human tissues, with the highest amounts detectable in testes. It has been proposed that tankyrase interferes with the binding of TRF1 to telomeres, which in turn has an effect on telomere length. Tankyrase co-localizes with TRF1 at the ends of human chromosomes in metaphase and interphase, and also resides at nuclear pore complexes and

centrosomes. Smith et al. reported that the gene for tankyrase is positioned at 17.6 cR_{10000} on human chromosome 8 with a LOD of 8.2 on the G3 map.

The molecular events involved in managing chromosome structure and regulating cell senescence are extremely complex. Each new protein found to participate in this process provides new opportunities for monitoring and intervening in some of the fundamental events of cell biology.

SUMMARY OF THE INVENTION

This invention provides a new human protein which is hereby designated Tankyrase II. This new protein shares three domains with the Tankyrase protein of Smith et al.: the ANK domain comprising 24 repeats of the ankyrin motif, the SAM domain thought to be involved in protein-protein interaction, and the PARP domain that is responsible for the poly(ADP-ribose) polymerase activity. Tankyrase II further comprises has a new domain at the N-terminal, designated the GC domain, which has no known homologs.

One of the embodiments of this invention is an isolated polynucleotide having at least about 30 consecutive nucleotides contained in a human Tankyrase II encoding sequence, or that is contained in plasmids deposited under Accession No. 203919, or that hybridizes under stringent conditions to a Tankyrase II encoding sequence, but does not consist of the encoding sequence for human Tankyrase I or other previously known structurally related proteins, such as those having PARP activity. Another embodiment of this invention is an isolated polynucleotide having at least 100 consecutive nucleotides that is at least 90% identical to a Tankyrase II sequence, or contained in the deposited plasmids, but not in λ-phage, Tankyrase I, or other previously known sequences. Certain polynucleotides of this invention encode a protein comprising a GC domain, a PARP domain, a SAM domain, or an ANK domain, or a protein that binds other telomere-associated proteins like TRF1, TRF2, TIN2, and Tankyrase I, or that ADP-ribosylates a target protein in the presence of NAD^+. Polynucleotides of this invention can be used to obtain the encoded polypeptide, or to determine other polynucleotides that encode Tankyrase II-like protein.

Another embodiment of this invention is an isolated polypeptide comprising a sequence of at least 10 consecutive amino acids that is contained in Tankyrase II, or is contained in the deposited plasmids, but is not contained in any previously known peptide sequence. Another embodiment of this invention is an isolated polypeptide comprising a sequence of at least 25 consecutive amino acids that is at least 90% identical to a Tankyrase II protein sequence, or a protein sequence encoded in the deposited plasmids. Certain polypeptides of this invention comprise a GC domain, a PARP domain, a SAM domain, or an ANK domain, or have activity for binding other telomere-associated proteins like TRF1, TRF2, TIN2, and Tankyrase I, or ADP-ribosylate a target protein in the presence of NAD^+.

A further embodiment of this invention is an isolated human Tankyrase II protein or fragment thereof, at least 10-fold higher in purity (or more) on a weight per weight basis than what occurs in natural sources.

Also embodied in this invention are polynucleotides encoding the polypeptides of this invention, and antibodies of any sort that bind specifically to the polypeptides of this invention. Some of the antibodies inhibit the catalytic activity of Tankyrase II; inhibit the binding of Tankyrase II to other telomere associated protein; or inhibit protein ribosylation mediated by Tankyrase II. Peptides can be obtained by expressing a polynucleotide of the invention in a suitable host cell. Also provided are means for obtaining any antibody of this invention, comprising immunizing an animal or contacting an immunocompetent particle with a polypeptide of this invention. Peptides of this invention can be isolated from a mixture by using an antibody as a specific adsorbant; conversely, antibodies of this invention can be isolated using a peptide epitope as a specific adsorbant.

A further embodiment of this invention is a method for ribosylating a target protein, comprising incubating the target protein with a peptide of this invention in the presence of NAD^+.

Assay methods of this invention include determining Tankyrase II binding activity by incubating with a peptide of this invention under conditions where the protein can bind the peptide specifically to form a complex, and then correlating any complex formed with the presence or amount of the protein in the sample. The protein that has Tankyrase II binding activity can optionally be TRF1, TRF2, TIN2, or Tankyrase I.

Another assay method of this invention is for screening a test compound to determine an ability to affect Tankyrase II activity, comprising incubating the compound with containing a peptide of this invention and a conjugate binding ligand, and determining any effect of the test compound on complex formation. Another such method comprises incubating a test compound with a peptide of this invention, a potential target protein, and NAD^+; then determining any effect of the test compound on the amount or rate of ribosylation of the target.

This invention also includes a method for modulating Tankyrase II expression in a cell, comprising contacting the cell with the polynucleotide of this invention such as an antisense polynucleotide, a ribozyme, or an inhibitory RNA under conditions where the polynucleotide can interfere with mRNA translation. Modulating Tankyrase II expression in turn is believed to modulate telomere length in the cell.

These and other embodiments of the invention will be apparent from the description that follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic depiction of Tankyrase II protein. The domains depicted are the GC domain (encoded by a gene segment rich in GC), the ANK domain, containing contains 24 ankyrin repeats thought to be involved in protein-protein interaction, the sterile alpha motif (SAM) domain, thought to be involved in cellular signaling, and the poly (ADP)-ribose polymerase (PARP) domain, with enzymatic activity for ribosylating target proteins such as TRF1.

Figures 2, 3, and 4 are sequence listings showing cDNA and amino acid sequence data for human Tankyrase II (SEQ. ID NOs: 1 to 6). The data from Figures 2-3 were obtained as described in Examples 1-4; the data from Figure 4 were obtained as described in Examples 6-7.

Figure 5 is a sequence listing comparing Tankyrase II (SEQ. ID NO:6) with its closest known intraspecies homolog, Tankyrase I (SEQ. ID NOs:8), at the protein level.

Figure 6 is a sequence listing comparing Tankyrase II (SEQ. ID NO:5) with Tankyrase I (SEQ. ID NO:7), at the cDNA level.

DETAILED DESCRIPTION OF THE INVENTION

This disclosure describes the newly discovered protein Tankyrase II. Polynucleotides, polypeptides, and antibodies related to Tankyrase II are provided and exemplified. The protein has enzymatic activity that causes ribosylation of proximal target proteins using NAD as substrate. Tankyrase II is thought to have binding activity for other telomere-associated proteins, which could become ribosylated targets of the enzyme. This in turn could play a role in the regulation of telomere length, thereby affecting the replicative capacity of the cell. The techniques and materials in this disclosure provide the means to model Tankyrase II activity in vitro, and provide a way to monitor and modulate Tankyrase II activity in vivo. Modulation of Tankyrase II activity may be used to regulate telomerase activity or telomere length.

Figure 1 shows the structurally distinct domains of Tankyrase II, which provide different functional features of tankyrase activity. There is a unique amino-terminal (GC) domain, followed by an ankyrin (ANK)

motif domain, a sterile alpha module (SAM) domain, and a carboxy-terminal poly(ADP) ribose polymerase (PARP) domain.

The ankyrin (ANK) domain of Tankyrase II contains 24 ankyrin repeats — a motif of about 33 residues found in a number of different proteins, and thought to act as modular adapters for heterologous protein-protein interactions (reviewed by Bennett et al., J. Biol. Chem. 267:8703, 1992; Bennett et al., and Michaely, TICB 2:127, 1992; Bork et al., Proteins: Structure, Function, & Genetics 17:363, 1993). A correlation has been observed between the number of ankyrin repeats and the nature of the protein-protein association. Ankyrin family members containing 24 ankyrin repeats bind cytoskeletal proteins such as tubulin and spectrin.

The sterile alpha motif (SAM) domain of Tankyrase II lies downstream from the ANK domain. SAM domains are found in signaling proteins such as transcription factors, serine/threonine protein kinases, and GTPases, (Stapleton et al., 1999, Nature Struct. Biol. 6:44-9; Thamos et al., 1999, Science 283: 833-36). SAM-containing proteins form hetero- and homo-dimers with other SAM-containing proteins that can regulate cellular signaling processes.

The carboxy-terminus of Tankyrase II is a domain homologous to other proteins with poly (ADP)-ribose polymerase activity, referred to as the PARP domain. Proteins that contain a PARP domain catalyze the addition of long branched chains of ADP-ribose to target proteins, using nicotinamide adenine dinucleotide (NAD^+) as a substrate (reviewed by Still et al., Genomics 62:533, 1999; de Murcia et al., Trends Biochem. Sci. 19:172, 1994; Lindahl et al., Trends Biochem. Sci. 20:405, 1995). The first such protein, PARP-1 (Adprt1) contains DNA-binding zinc fingers, a nuclear localization sequence, and an automodification domain. PARP-1 binds to nicked DNA, and is thought to play a role in chromosomal damage repair (P.A. Jeggo, Current Biol. 8:R49, 1998). PARP-2 (AdprtL2) is a homologous protein with ribosylation activity that also binds damaged DNA (Amé et al. J. Biol. Chem. 274:17860, 1999). VPARP is a related protein that ribosylates major vault protein in the mammalian ribonucleoprotein complex (Kickhoefer, J. Cell Biol. 146:917, 1999). Other members of the PARP family include Tankyrase I and AdprtL1 (Still et al., supra).

The presence of the ANK, SAM and PARP domains in Tankyrase II suggests that Tankyrase II plays a role in intercellular or intracellular communication (e.g., signal transduction), possibly in conjunction with proteins involved in DNA repair pathways or maintenance of telomeres. Ribosylation can also play an important role in how Tankyrase II regulates other proteins involved in telomere management. Ribosylation of telomere-associated proteins may result in them leaving the telomere, potentially modulating the activity of telomerase reverse transcriptase, and thereby affecting telomere length and replicative capacity of the cell.

The SAM, PARP and ANK domains of Tankyrase II are homologous to counterpart domains in the Tankyrase I protein. However, the N-terminal domains appear to have no homology. Tankyrase I has a 180-residue HPS domain, so called for the abundance of histidine, proline, and serine residues. In contrast, Tankyrase II has a substantially different amino acid composition, encoded by a highly GC-rich gene sequence. This N-terminal domain of Tankyrase II will be referred to in this disclosure as the "Divergent" or "GC" domain.

There is a relationship between the attainment of a critical telomere length in dividing somatic cells and DNA damage, and both processes lead to cell cycle arrest and the activation of gene expression pathways. Thus, Tankyrase II may communicate with a subset of the signaling molecules in DNA repair processes to initiate the specific arrest and gene activation pathways of cellular senescence. Notably, Tankyrase I has been demonstrated to ribosylate both itself and TRF1 (Smith et al., 1998, supra), resulting in a reduction of the ability of TRF1 to bind telomeric DNA. The link between telomere structure and DNA repair is supported by the observation that p53- and ATM (ataxia telangiectasia mutated) dependent apoptosis is induced by telomeres with attenuated TRF2 function (Karlseder et al., 1999, Science 283:1321-1325).

The structural features of Tankyrase II indicate that it binds to nuclear and cell proteins, and is involved in intercellular or intracellular cell signaling that affect telomere structure and metabolism. Compositions and treatments that modulate Tankyrase II expression or function are likely to be of therapeutic benefit for cancer, disorders associated with replicative senescence, and other conditions associated with perturbations of telomerase activity or telomere length.

Definitions

The term "polynucleotide" as used in this disclosure refers to a polymeric form of nucleotides of any length. Included are genes and gene fragments, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA and RNA, nucleic acid probes, and primers. Also included are nucleotide analogs, including but not limited to thiol-derivatized nucleosides (U.S. Patent 5,578,718), oligonucleotides with modified backbones (U.S. Patent Nos. 5,541,307 and 5,378,825), and peptide nucleic acids (U.S. Patent No. 5,786,461). The term polynucleotide, as used in this disclosure, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention that is a polynucleotide encompasses both a double-stranded form, and each of the two complementary single-stranded forms known or predicted to make up the double-stranded form.

The term "oligonucleotide" is reserved for polynucleotides of no more than 100 bases in length, and may or may not be accompanied with an antisense strand, depending on context. Oligonucleotides are often used as probes in specific hybridization reactions, or as primers in amplification reactions.

When comparison is made between polynucleotides for degree of identity, it is implicitly understood that complementary strands are easily generated, and the sense or antisense strand is selected or predicted that maximizes the degree of identity between the polynucleotides being compared. Percentage of sequence identity is calculated by first aligning the polynucleotide being examined with the reference counterpart, and then counting the number of residues shared between the sequences being compared as a percentage of the region under examination. No penalty is imposed for the presence of insertions or deletions, but insertions or deletions are permitted only where clearly required to readjust the alignment. The percentage is given in terms of residues in the sequence being examined that are identical to residues in the comparison or reference sequence. Particularly desirable polynucleotide sequences preserve at least one function of the prototype. By way of example and depending on context, the function preserved may include an ability to hybridize with a target sequence, the function of a polypeptide it may encode, or (for certain gene targeting vectors) the ability to facilitate homologous recombination or gene inactivation. An example of an algorithm suitable for finding homologous sequences and determining percent sequence identity is the BLAST algorithm, (Altschul et al., 1990, J. Mol. Biol. 215:403, 1990; Karlin et al., Proc. Natl. Acad. Sci. USA 90:5873, 1993), available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/entrez).

Polynucleotide sequences are said to be in a "non-natural arrangement" when they are joined together or interposed with another sequence in an arrangement not found in nature.

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding can occur by Watson-Crick base pairing, Hoogsteen binding, triplex formation, or complexing in any other sequence-specific manner. A hybridization reaction will, on occasion, be a step in a more extensive process, such as part of PCR amplification. Hybridization reactions can be performed under conditions of different "stringency". Conditions that increase the stringency of a hybridization reaction are widely known (see e.g., Sambrook et al., *infra*). Examples of conditions in order of increasing stringency: Incubation temperatures of 25°C, 37°C, 50°C, and 68°C; buffer concentrations of 10 x SSC, 6 x SSC, 1 x SSC, 0.1 x SSC (where SSC is

0.15 M NaCl and 15 mM citrate buffer, pH 7.2) and their equivalent using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 min to 24 h; 1, 2, or more washing steps; wash incubation times of 1, 5, or 15 min; and wash solutions of 6 x SSC, 1 x SSC, 0.1 x SSC, or deionized water. Typical conditions of high stringency for the binding of a probe of about 100 base pairs and above is a hybridization reaction at 65°C in 2 x SSC, followed by repeat washes at 0.1 x SSC — or the equivalent combination of solvent and temperature conditions for the particular nucleic acids being studied.

A "hybrid" of polynucleotides, or a "complex" formed between any two or more components in a biochemical reaction (such as antibody and antigen), refers to a duplex or higher-order complex that is sufficiently long-lasting to persist between its formation and subsequent detection.

A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. "Operatively linked" refers to an operative relationship between genetic elements, in which the function of one element influences the function of another element. For example, an expressible encoding sequence may be operatively linked to control element that permit transcription and translation.

The terms "polypeptide", "peptide" and "protein" are used interchangeably in this disclosure to refer to polymers of amino acids of any length. The polymer may comprise modified amino acids, it may be linear or branched, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, and/or phosphorylation.

Percentage of sequence identity is calculated for polypeptides by first aligning the polypeptide being examined with the reference counterpart or prototype, and then counting the number of residues shared between the sequences being compared as a percentage of the region under examination. No penalty is imposed for the presence of insertions or deletions, but insertions or deletions are permitted only where clearly required to readjust the alignment. The percentage is given in terms of residues in the sequence being examined that are identical to residues in the comparison or reference sequence. Where substitutions are made, conservative substitutions (in which one amino acid is substituted by another with similar charge, size, hydrophobicity, or aromaticity) are typically better tolerated. Desirable sequences preserves the function of the prototype: for example, the enzymatic activity, the binding of specific substrates, and the binding of specific antibody as detectable in a standard competition inhibition immunoassay. In certain embodiments, the identity may exist over a region that is at least about 10, 20-25, or 50-100 amino acids in length.

The term "antibody" as used in this disclosure refers to both polyclonal and monoclonal antibody of any species. The ambit of the term deliberately encompasses not only intact immunoglobulin molecules, but also such fragments and genetically engineered derivatives of immunoglobulin molecules (including humanized forms) that may be prepared by techniques known in the art, and retaining the binding specificity of the antigen binding site.

An "immunogenic" compound or composition is capable of stimulating production of a specific immunological response when administered to a suitable host, usually a mammal.

An "isolated" polynucleotide, polypeptide, protein, antibody, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially obtained from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Enrichments by 2, 10, 100, and

1000 fold achieve improved degrees of purification. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis or recombinant expression. An "isolated" cell is a cell that has been separated from the organism in which it was grown.

A polynucleotide used in a reaction, such as a probe used in a hybridization reaction or a vector used in gene targeting is referred to as "specific" or "selective" if it hybridizes or reacts with the intended target more frequently, more rapidly, or with greater duration than it does with alternative substances. Similarly, a polypeptide is referred to as "specific" or "selective" if it binds an intended target, such as a ligand, hapten, substrate, antibody, or other polypeptide more frequently, more rapidly, or with greater duration than it does to alternative substances. An antibody is referred to as "specific" or "selective" if it binds via at least one antigen recognition site to the intended target more frequently, more rapidly, or with greater duration than it does to alternative substances.

General Techniques

Unless otherwise noted, the practice of this invention can be carried out by employing standard techniques of genetic engineering, protein manipulation, and cell culture. Textbooks that describe standard laboratory techniques include *"Molecular Cloning: A Laboratory Manual"*, 2nd Ed. (Sambrook et al., 1989); *"Oligonucleotide Synthesis"* (M.J. Gait, ed., 1984); *"Animal Cell Culture"* (R.I. Freshney, ed., 1987); the series *"Methods in Enzymology"* (Academic Press, Inc.); *"Gene Transfer Vectors for Mammalian Cells"* (J.M. Miller & M.P. Calos, eds., 1987); *"Current Protocols in Molecular Biology"* and *"Short Protocols in Molecular Biology, 3rd Edition"* (F.M. Ausubel et al., eds., 1987 & 1995); and *"Recombinant DNA Methodology II"* (R. Wu ed., Academic Press 1995). Techniques used in raising, purifying and modifying antibodies, and the design and execution of immunoassays, are described in *Handbook of Experimental Immunology* (D.M. Weir & C.C. Blackwell, eds.); *The Immunoassay Handbook* (Stockton Press NY, 1994); and R. Masseyeff, W.H. Albert, and N.A. Staines, eds., *Methods of Immunological Analysis* (Weinheim: VCH Verlags GmbH, 1993).

Polynucleotides

The polynucleotides of this invention include those containing nucleotide sequences which are found within the Tankyrase II DNA sequence, shown in SEQ. ID NOs:1, 3, and 5. Further sequence for Tankyrase II gene can be obtained by employing standard sequencing techniques known in the art to the phage plasmids deposited in support of this application.

Also included in this invention are polynucleotides that are from naturally occurring allelic variants, synthetic variants, and homologs of Tankyrase II with a percentage of residues identical to the Tankyrase II cDNA or gene sequence, determined as described above. It is understood that substitutions, insertions, and deletions can be accommodated within a polynucleotide sequence without departing from the spirit of this invention. In certain embodiments, the polynucleotide sequences are at least about 80% , 90%, 95%, or 98% identical to a sequence or part of a sequence exemplified in this disclosure; in order if increasing preference. In other embodiments, the polynucleotide sequences are 100% identical to a reference sequence or a fragment thereof. The length of consecutive residues in the identical or homologous sequence compared with the exemplary sequence can be at least about 15, 30, 50, 75, 100, 200 or 500 residues in order of increasing preference, up to the length of the entire clone, gene, or sequence.

This invention includes polynucleotides that are uniquely related to the prototype polynucleotide sequences, in comparison with other sequences that may be present in a sample or reaction mixture of interest. By way of example, probes of at least about 100 consecutive nucleotides that are at least 90% or 80% identical to a reference sequence may be specific, and a probe of at least about 500 or 2000 consecutive nucleotides

may be specific if at least 90%, 80%, 70%, or even 60% identical with the reference sequence, depending on hybridization conditions, as explained below. On occasion, such nucleotides can be divided into halves (about nucleotides 1-2137 and 2138-4275), or quarters (about 1-1068; 1069-2137; 2138-3206; 3207-4275), and still retain their specificity. Nucleic acid molecules comprising specified lengths of consecutive nucleotides can be selected from any of these regions. It will also be recognized that for some purposes such as hybridization reactions, a specific polynucleotide sequence will readily accommodate deletions from the 5' or 3' end of either strand of say, 15, 25, or even 50 nucleotides without compromising function. Internal deletions may also be tolerated.

Of particular interest are polynucleotides that are distinct from polynucleotides encoding Tankyrase I, and other proteins containing ANK, PARP, or SAM domains. In certain embodiments of the invention, polynucleotides are distinct from one or more previously known EST sequences, such as those in GenBank Accession Nos. R64714, AA244138 (SEQ. ID NO:9), AA244137, AA307492, H11865, H17748, N57467, R06946, AI247608, R06902, AI247608, H11505, H17635, N29528, AA088990 AI426537, and AW157349 (SEQ. ID NO:10), and those listed elsewhere in this disclosure. A polynucleotide of this invention can be "distinct" from other polynucleotides because of an internal sequence difference (a substitution, deletion, or insertion), or because it is defined to encompass additional sequence at either end. Also included in the invention are recombinant or synthetic polynucleotides in which a Tankyrase II-like sequence is linked to a heterologous sequence to form: for example, a heterologous promoter in an expression vector, or a selectable marker such as *neo* in a targeting vector.

The polynucleotides of this invention can be in the form of an expression vector, in which the encoding sequence is operatively linked to control elements for transcription and translation in a prokaryotic or eukaryotic host cell of interest. A variety of suitable vectors and their design and manufacture are known in the art. Vector systems of interest include but are not limited to those based on retroviruses, adenoviruses, adenoassociated viruses, herpes viruses, SV40, papilloma virus, Epstein Barr virus, vaccinia virus, lenti virus, and Semliki Forest virus.

Particular polynucleotides of this invention are useful for producing polypeptides of interest, as nucleotide probes and primers, and as targeting vectors for genetic knockouts. Further description of the characteristics of such constructs are provided elsewhere in this disclosure.

Preparation

The polynucleotides of this invention can be prepared by any suitable technique in the art. Using the data provided in this disclosure or deduced from the deposited plasmids, sequences of less than ~50 base pairs are conveniently prepared by chemical synthesis, either through a commercial service or by a known synthetic method, such as the triester method or the phosphite method. A suitable method is solid phase synthesis using mononucleoside phosphoramidite coupling units (Hirose et al., *Tetra. Lett.* 19:2449-2452, 1978; U.S. Patent No. 4,415,732).

For use in antisense therapy, polynucleotides can be prepared synthetically that are more stable for the pharmaceutical preparation for which they are intended. Non-limiting examples include thiol-derivatized nucleosides (U.S. Patent 5,578,718), oligonucleotides with modified backbones (U.S. Patent Nos. 5,541,307 and 5,378,825). Also of interest in the context of antisense constructs are peptide nucleic acids. Prototype PNA have an achiral polyamide backbone consisting of N-(2-aminoethyl)glycine units, to which purine and pyrimidine bases are linked, for example, by way of a methylene carbonyl linker. PNAs are nuclease and protease resistant, and the uncharged nature of the PNA oligomers enhances the stability of PNA-nucleotide duplexes, thereby blocking transcription or translation. Uptake into cells can be enhanced by conjugating to

lipophilic groups incorporating into liposomes, and introducing an amino acid side chain into the PNA backbone. See Soomets et al., Front. Biosci. 4:D782, 1999; U.S. Patents 5,539,082, 5,766,855, 5,786,461, and International Patent Application WO 8/53801.

Polynucleotides of this invention can also be obtained by PCR amplification of a template with the desired sequence. Oligonucleotide primers spanning the desired sequence are annealed to the template, elongated by a DNA polymerase, and then melted at higher temperature so that the template and elongated oligonucleotides dissociate. The cycle is repeated until the desired amount of amplified polynucleotide is obtained (U.S. Patent Nos. 4,683,195 and 4,683,202). Exemplary primers are shown in Table 1. Suitable templates include the plasmids deposited in support of this application, and cDNA libraries for cells expressing Tankyrase II. Encoding sequences, intron sequences, and upstream or downstream sequences for Tankyrase II can be obtained from a human genomic DNA library.

TABLE 1: Exemplary primers for amplifying Tankyrase II sequences

	Forward & Reverse Primers		Function
UTANKII-32:	5'-TCCAGAGGCTGGTGACCCCTGA-3'	SEQ. ID NO: 11	Amplifies entire ANK domain
LTANKII-37:	5'-TTGAACTAACTACTGAAGA -3'	SEQ. ID NO: 12	
UTANKII-38:	5'-CTGTCTTCAGTAGTTAGTTCA-3'	SEQ. ID NO: 13	Amplifies entire SAM domain
LTANKII-39:	5'-GTTACAAACCTTCTGAATCT-3'	SEQ. ID NO: 14	
UTANKII-40:	5'-GAAAGATACACTCACCGGA-3'	SEQ. ID NO: 15	Amplifies entire PARP domain
LTANKII-41:	5'-TAGGGTTCAGTGGGAATTAG-3'	SEQ. ID NO: 16	
gt11-5':	5'GACTCCTGGAGCCCGTCA-3'	SEQ. ID NO: 17	Amplifies λ 11L-1-1 cDNA insert
gt11-3':	5'-GGTAGCGACCGGGCGTCA-3'	SEQ. ID NO: 18	

Production scale amounts of large polynucleotides are most conveniently obtained by inserting the desired sequence into a suitable cloning vector and reproducing the clone. Techniques for nucleotide cloning are given in Sambrook, Fritsch & Maniatis (supra) and in U.S. Patent No. 5,552,524. Exemplary cloning and expression methods are illustrated in Examples 1 and 2, below. Polynucleotides can be purified by standard techniques in nucleic acid chemistry, such as phenol-chloroform extraction, agarose gel electrophoresis, and other techniques known in the art, adapted according to the source of the polynucleotide.

Assessment and use of the polynucleotides

Polynucleotides of this invention can be used to identify Tankyrase II nucleotide sequences in a sample of interest for research, diagnostic evaluation, or any other purpose. Generally, this will involve preparing a reaction mixture in which a sample suspected of containing an Tankyrase II-related sequence is contacted with a polynucleotide of this invention under conditions that permit the polynucleotide to hybridize specifically with the compound being tested for, detecting any stable hybrids that form, and correlating the hybrids with the presence of a Tankyrase II related sequence in the sample. The formation of stable hybrids can be detected by any suitable method known in the art. For example, the probe sequence with a detectable label such as a radioisotope, a chromophore, or a hapten such as avidin to which an signaling reagent can be attached. Alternatively, the reagent polynucleotide can be a primer for an amplification reaction in which the amount of product produced correlates with the formation of specific hybrids.

The specificity of the probe or primer, and the stringency of hybridization conditions are both chosen with a view to facilitating detection of sequences of interest, while diminishing false positive reactions. Thus, when it is important to distinguish between Tankyrase II sequences from Tankyrase I sequences, particularly when using sequence outside the GC domain, then stringency conditions should be high, and the reagent polynucleotide should be nearly identical to the sequence being tested for. Conditions can be determined empirically so that the reagent polynucleotide will hybridize with the Tankyrase II sequence being tested for but not with other sequences that might be present in the sample of interest. In other instances, assays for Tankyrase II are conducted on samples where Tankyrase I is not present, or where it is desirable to test for Tankyrase I and Tankyrase II together. In these instances, the capability of the probe to cross-hybridize with Tankyrase I is not a hindrance, and may provide certain advantages.

Polynucleotides of this invention can also be used to inhibit the transcription or translation of Tankyrase II in target cells. Such polynucleotides can be in the form of antisense constructs, which in some embodiments binds to Tankyrase II mRNA and prevent translation. Other polynucleotides of this invention are ribozymes having a substrate (Tankyrase II mRNA) binding portion, and an enzymatic portion with endonuclease activity that cleaves the substrate. Design and use of ribozymes is described generally in U.S. Patent Nos. 4,987,071, 5,766,942, 5,998,193, and 6,025,167. The modulation of Tankyrase II expression using ribozyme constructs is embodied in this invention.

This invention also includes interfering RNA (RNAi) complexes. The structure and activity of RNAi is reviewed by Bosher et al. (Nature Cell Biol. 2:E31, 2000) and C.P. Hunter (Curr. Biol. 9:R440, 1999). The RNAi complexs of this invention comprise double-stranded RNA comprising Tankyrase II sense and antisense polynucleotides (optionally in a hairpin configuration) that specifically inhibits translation of mRNA encoding Tankyrase II and Tankyrase II-like proteins. Also contemplated are polynucleotides that bind to duplex Tankyrase II sequences to form a triple helix-containing nucleic acid, blocking expression at the transcription level (Gee et al., in Huber and Carr, 1994, Molecular and Immunologic Approaches, Futura Publishing Co.; Rininsland et al., 1997, Proc. Natl. Acad. Sci. USA 94:5854, 1997).

This invention also encompasses polynucleotides that encode polypeptides of interest. Characteristics of the polypeptides of this invention are described in the section that follows. For polypeptides that are fragments of naturally occurring Tankyrase II, there will be a corresponding naturally occurring polynucleotide encoding sequence. Those skilled in the art will recognize that because of redundancies in the amino acid code, any polynucleotide that encodes a peptide of interest can be used in a translation system to produce the peptide. Except where otherwise required, all possible codon combinations that translate into the peptide sequence of interest are included in the scope of the invention.

Polypeptides

The polypeptides of this invention include those that comprise amino acid sequences encoded within any of the polynucleotides of this invention, exemplified by SEQ. ID NO:6 and its subfragments. Also included in this invention are polypeptides containing Tankyrase II like sequence that is from naturally occurring allelic variants, synthetic variants, and homologs of Tankyrase II with a percentage of residues identical to the Tankyrase II protein, calculated as described elsewhere in this disclosure.

It is understood that substitutions, insertions, and deletions can be accommodated within a protein sequence without departing from the spirit of this invention. Conservative substitutions are typically more tolerable, such as the substitution of charged amino acids with amino acids having the same charge, or substituting aromatic or lipophylic amino acids with others having similar features. Certain peptides of this invention are 60%, 80% , 90%, 95%, or 100% identical to one of the sequences exemplified in this disclosure;

in order of increasing preference. The length of the identical or homologous sequence compared with the prototype polypeptide can be about 7, 10, 15, 25, 50 or 100 residues in order of increasing preference, up to the length of the entire protein.

This invention includes polypeptides that are uniquely related to the prototype sequences, in comparison with other sequences that may be present in a sample or reaction mixture of interest. By way of example, peptides of at least about 10 consecutive amino acids that are at least 90% or 80% identical to a reference sequence, or wherein the rest of the peptide contains only conservative substitutions, may uniquely identify the peptide in terms of functional or antigenic characteristics. A peptide of at least about 25, 100, or 300 consecutive amino acids may be specific if at least 90%, 70%, 60%, or even 50% identical with the reference sequence. Longer peptides can be divided into halves (amino acids 1-584 and 585-1166 of SEQ. ID NO:6), or quarters (amino acids 1-292; 293-584; 585-876; 877-1166) and still retain one or more of their functional activities — such as ribosylation of target proteins, and the binding to conjugate peptides through the Tankyrase II ANK and SAM domains. Peptides from the region encoded by nucleotides 1-283 of SEQ. ID NO:5 are also of interest. It will be recognized that for some purposes such as reactions with antibody or the contact region on an opposing protein, a specific polypeptide sequence will readily accommodate deletions from the N- or C- end, say, of 3, 5, or even 10 amino acid residues.

Certain peptides of this invention are distinct from peptides previously known: These include human Tankyrase I proteins, and other proteins comprising PARP, SAM, and ANK domains. In certain embodiments, polypeptides of the invention are distinct from predicted amino acid sequences encoded in one or more previously known polynucleotide sequences, such as those cited at other places in this disclosure. A polypeptide of this invention can be "distinct" from other polypeptides because of an internal sequence difference (a substitution, deletion, or insertion), or because it is defined to encompass additional sequence at either end. Also included in the invention are artificially engineered fusion proteins in which a Tankyrase II like sequence is linked to a heterologous sequence which modulates Tankyrase II activity, provides a complementary function, acts as a tag for purposes of labeling or affinity purification, or has any other desirable purpose.

Preparation

Short polypeptides of this invention can be prepared by solid-phase chemical synthesis. The principles of solid phase chemical synthesis can be found in Dugas & Penney, Bioorganic Chemistry, Springer-Verlag NY pp 54-92 (1981), and U.S. Patent No. 4,493,795. Automated solid-phase peptide synthesis can be performed using devices such as a PE-Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City CA).

Longer polypeptides are conveniently obtained by translation in an in vitro translation system, or by expression in a suitable host cell. To produce an expression vector, a polynucleotide encoding the desired polypeptide is operably linked to control elements for transcription and translation, and then transfected into a suitable host cell. Expression may be effected in prokaryotes such as E. coli, eukaryotic microorganisms such as the yeast Saccharomyces cerevisiae, or higher eukaryotes, such as insect or mammalian cells. Control elements such as the promoter are chosen to permit translation at an acceptable rate under desired conditions. A number of expression systems suitable for producing the peptides of this invention are described in U.S. Patent No. 5,552,524. Expression cloning is available from such commercial services as Lark Technologies, Houston TX.

Following production, the protein is typically purified from the producing host cell by standard methods in protein chemistry in an appropriate combination, which may include ion exchange chromatography, affinity

chromatography, and HPLC. Expression products are optionally produced with a sequence tag to facilitate affinity purification, which can subsequently be removed by proteolytic cleavage.

Also contemplated are Tankyrase II protein isolated from human biological samples, including tissue samples and cultured cell lines, tracking activity on the basis of functional assays and/or immunoassays provided below. Antibody to Tankyrase II described in the following section can be used in immunoaffinity or immunoprecipitation techniques to enrich Tankyrase II from biological samples. If desired, fragments can be made from whole Tankyrase II by chemical cleavage (e.g., using CNBr), or enzymatic cleavage (using trypsin, pepsin, dispase, V8 protease, or any other suitable endopeptidase or exopeptidase). Enrichment of peptides and proteins of this invention from natural or synthetic sources provides a purity of 10-fold, 100-fold, 1000-fold, or 10,000-fold higher than what is found in nature, in terms of a weight to weight ratio of Tankyrase II peptide to other proteins in the sample mass.

Assessment and use of the polypeptides

Polypeptides of this invention can be used for a number of purposes, including but not limited to the characterization of telomerase function and how it is regulated, assays for proteins and nucleotide sequences to which Tankyrase II binds, the identification of new proteins with Tankyrase II binding activity that may play a role in maintaining telomere length, replicative capacity, apoptosis, chromosome packing, or gene expression, and the obtaining of antibody specific for Tankyrase II.

Subregions of Tankyrase II and homologs can be assessed for function based on the known domain structure of Tankyrase II, and employed according to the role they play in the activity of the whole molecule.

A putative PARP domain in a Tankyrase II homolog can be identified on the basis of sequence similarity, since a high degree of conservation with Tankyrase II PARP and other proteins with PARP domains (e.g., Tankyrase I, PARP-1 and PARP-2), especially over critical conserved residues, correlates with ribosylation activity. Residues of Tankyrase II thought to play an important role in the enzymatic activity are shown in Table 2. Functional assays for poly(ADP-ribose) polymerase can be conducted by incubating the putative PARP-containing peptide with a target protein (such as the Tankyrase II ANK and domains), or TRF1, in the presence of nicotinamide adenine dinucleotide (NAD^+), or an analog labeled with a radioisotope such as ^{32}P or ^{33}P, biotin, or a fluorescent group. ADP-ribosylation can be monitored by incorporation of the label into the protein phase, by a change of size of the target protein (measurable, for example on a protein gel), or by detection of ADP ribose polymers on the target (for example, using commercially available antibody specific for ADP-ribose polymers, by digestion with glycohydrolases, or by physical-chemical mechanisms, such as mass spectrometry). Known PARP inhibitors like 3 amino-benzamide (3AB) can be used to verify the specificity of the assay. A number of other assays for rapid detection of poly(ADP-ribose) polymerase activity have been described. See Sallmann et al., Mol. Cell Biochem. 185:199, 1998; Simonin et al., Anal. Biochem. 195:226, 1991; Shah et al., Anal. Biochem 232:251, 1995. Peptides with confirmed PARP activity can then be used as a reagent to ADP-ribosylate protein targets of interest.

A putative SAM domain can also be identified on the basis of sequence similarity with the sterile alpha motif domain in other proteins (Tankyrase II, Tankyrase I, EphB2 receptor, and others reviewed by Stapleton et al., Nature Struct. Biol. 6:44, 1999). The SAM domain has been implicated in forming homodimers and heterodimers with other SAM-containing proteins (Stapleton et al., op. cit.; and Kyba et al., Dev. Genet. 22:74, 1998; Thanos et al., Science 283:833, 1999). Thus, putative SAM domains can be screened functionally in dimerization reactions: either with themselves, or with SAM domains from other proteins with known heterodimerization activity. Dimerization can be detected in an equilibrium system (e.g., using a biosensor), or in a separation system (e.g., by gel filtration chromatography or in a gel shift experiment). Dimerization can

also be detected in a reporter gene assembly — for example, where a conjugate binding site on another protein is fused to a DNA-binding peptide, and the putative SAM domain is fused to a trans-activator. These constructs are then transfected into a cell comprising a reporter gene (such as Lac Z), which signals proximity of the trans-activator, indicating binding between the two peptides. Peptides with confirmed SAM activity according to any of these assays can then be used in turn as reagents in a dimerization assay to detect or quantitate Tankyrase II or other proteins with a SAM domain. They can also be used to inhibit Tankyrase II activity by competition at the SAM binding site.

Putative ANK and GC domains can also be identified on the basis of sequence similarity to corresponding domains in Tankyrase II. In addition, ANK domains characteristically have a number of tandem repeats about 33 amino acids long. Dozens of proteins containing anywhere from one to dozens of ANK repeats are known. Michaely et al. (Trends Cell Biol. 2:127, 1992) report the consensus sequence as

```
-XGXTPLHLAARXGHVEVVKLLLDXGADVNAXTK-
     A  I SQ NNLDIAEV  K   NPD  D
        V  K   T M R   Q   SI   N
                       E
```

SEQ. ID NO:19

ANK repeats generally are implicated in protein-protein binding, and the ANK domain in Tankyrase I is responsible for the binding of TRF1. Tankyrase II is believed to have binding activity for several proteins involved in telomere regulation and other aspects of chromosome management. Such proteins include TRF1, TRF2, TIN2, and Tankyrase I, which are all proteins known to interact in the management of telomeres. Peptide fragments and homologs of Tankyrase II can be tested for binding to such proteins, and those showing activity can in turn be used to assay TRF1, TRF2, TIN2, or Tankyrase I. The general format of such an assay comprises incubating a sample suspected of containing the protein with Tankyrase II binding activity with a peptide of this invention under conditions where the protein can bind the peptide to form a complex, and correlating any complex formed with the presence or amount of Tankyrase II binding activity in the sample. In a similar fashion, fragments and homologs of Tankyrase II can be tested for binding to polynucleotides having particular sequences, such as the tandem repeats that are characteristic of telomeres. Since Tankyrase II binds and ribosylates telomere-associated proteins, fragments and homologs of Tankyrase II can also be tested for modulation of telomere length. Cells are transfected with an expression vector for the fragment or homolog, and the effect on telomere length is measured by a suitable method, such as the assays described in U.S. Patent Nos. 5,707,795, 5,741,677, and 5,834,193.

A systematic approach can be used to determine functional regions and homologs of Tankyrase II according to any of these assays. For example, the viability of an assay system is confirmed on the intact Tankyrase II protein; then a series of nested fragments is tested to determine the minimum fragment that provides the same activity. Similarly, amino acid substitutions can be introduced into the sequence until the activity is ablated, thereby determining what residues are critical for functional activity.

Peptides of this invention can also be used for the preparation and testing of antibodies against Tankyrase II, for the testing of other compounds for Tankyrase II binding activity, and for the screening of potential Tankyrase II modulators. These procedures are detailed further on in this disclosure.

Dominant Negative Mutants

Based on the sequence data provided in this disclosure, someone skilled in the art will be able to develop dominant negative polypeptide mutants of Tankyrase II, and polynucleotides that encode them. These mutants may be used to inhibit the function of Tankyrase II in a cell or reaction mixture. The production of

dominant negative mutants entails deleting or mutating an important functional element of the native Tankyrase II. For example, functional mutation or deletion of the ANK domain may produce peptides that do not bind to TRF1 or TRF2, but retain SAM binding activity. Conversely, a functional mutation or deletion of the SAM domain may produce peptides are deficient in binding to proteins such as TRF1 or TRF2, but still have the ribosylation activity of PARP. A functional mutation or deletion of the PARP domain (for example, mutation of all or a subset of the residues from the Tankyrase II C-terminus to alanine), may result in a peptide that binds Tankyrase II associated proteins, but does not have any ribosylation activity.

Muteins with point mutations can also be obtained. Specifically, amino acids thought to be critical for the activity of the domain could be changed to a neutral amino acid such as alanine, and then reassayed for functional activity. For Tankyrase II, mutations that may abolish ribosylation activity are changes to His (position 1031), Gly (1032), Gly (1058), Tyr (1060), Tyr (1071), and Glu (1138).

TABLE 2: Critical Residues for PARP Activity in Tankyrase II

ERYTHRRKEV	SEENHNHANE	RMLFHGSPFV	NAIIHKGFDE	RHAYIGGMFG
AGIYFAENSS	KSNQXVYGIG	GGTGCPVHKD	RSCYICHRQL	LFCRVTLGKS
FLQFSAMKMA	HSPPGHHSVT	GRPSVNGLAL	AEYVIYRGEQ	AYPEYLITYQ
IMRPEGMVDG				SEQ. ID NO:20

Screening for other Tankyrase II binding proteins

Those skilled in the art will readily appreciate that the assays described earlier in this section can be adapted to screen for other proteins that may be involved in telomere regulation, cell proliferative capacity, senescence, and apoptosis. The Tankyrase II domains ANK and SAM have characteristic features of protein binding molecules, and can be used to identify binding partners for Tankyrase II by incubating with a candidate compound under conditions suitable for binding, typically a physiological isotonic buffer containing any necessary cofactors that may promote transmolecular interaction. The formation of binding complexes with a candidate binding partner that is demonstrably specific (by virtue of being higher affinity than the binding of other candidate compounds) correlates with binding activity for Tankyrase II. Positive controls include peptides or proteins which have binding activity for Tankyrase II, while negative controls include ubiquitous and generally unreactive compounds, such as albumin. Candidates likely to screen positive for Tankyrase II binding include fragments and homologs related to telomere-associated proteins such as TRF1.

This type of conjugate binding assay can be conducted in several different formats. For example, Tankyrase II containing protein complexes can be isolated from human tissues or cell lines, for example, by tracking Tankyrase II through standard protein purification regimens by way of ribosylation activity or Tankyrase II antibody binding. In a similar approach, natural sources of Tankyrase II are solubilized in a suitable buffer, and Tankyrase II complexes are immunoprecipitated. The conjugate binding partner is then recovered from the complex, and characterized by physical and chemical criteria (such as apparent molecular weight determined by SDS gel electrophoresis), amino acid sequencing, or binding assays with Tankyrase II domains. In another example, Tankyrase II is labeled with a traceable substituent, such as biotin, a fluorescent group, an enzyme, a radioisotope, or a peptide group (e.g., FLG, HA, myc, or an immunoreactive peptide

sequence), and then combined in a reaction solution with an isolated candidate binding partner, or with a mixture of components (such as a cell extract) in which compounds with Tankyrase II binding activity may be found. Formation of complexes with the labeled Tankyrase II is then detected (for example, by gel shift techniques or immunoprecipitation), and correlated with binding activity for Tankyrase II.

Another format is a coexpression system, using Tankyrase II, a Tankyrase II fragment, or a Tankyrase II homolog as bait. For example, a yeast two-hybrid screen system is employed, in which a Tankyrase II encoding sequence is fused to one part of the expression system, and a library of candidate binding partners is fused to the complementary component needed for expression of the marker. Cloned cells that express the activity of the marker contain an insert that comprises the encoding sequence for a Tankyrase II binding partner. Yeast two-hybrid screen systems are described generally in Bianchi et al., EMBO J., 16:1785, 1997. Reagents and suitable libraries (e.g., human fetal liver cDNA transformants) are commercially available from Clontech, Palo Alto CA.

Antibodies

Antibody molecules of this invention include those that are specific for any novel peptide encompassed in this disclosure. These antibodies are useful for a number of purposes, including assaying for the expression of Tankyrase-II, and purification of Tankyrase-II peptides by affinity purification.

Polyclonal antibodies can be prepared by injecting a vertebrate with a polypeptide of this invention in an immunogenic form. If needed, immunogenicity of a polypeptide can be enhanced by linking to a carrier such as KLH, or combining with an adjuvant, such as Freund's adjuvant. Typically, a priming injection is followed by a booster injection is after about 4 weeks, and antiserum is harvested a week later. If desired, the specific antibody activity can be further purified by a combination of techniques, which may include Protein-A chromatography, ammonium sulfate precipitation, ion exchange chromatography, HPLC, and immunoaffinity chromatography using the immunizing polypeptide coupled to a solid support. Antibody fragments and other derivatives can be prepared by standard immunochemical methods, such as subjecting the antibody to cleavage with enzymes such as papain, pepsin, or trypsin.

Any unwanted cross-reactivity can be removed by treating the polyclonal antibody mixture with adsorbants made of those antigens attached to a solid phase, and collecting the unbound fraction. Contaminating activity against other proteins containing ANK, SAM, or PARP domains, or against Tankyrase I, or against Tankyrase-II from other species, can all be removed by adsorption if such cross-reactivity would interfere with the intended use of the antibody. Specificity of the original antisera can be improved to start with, by immunizing with peptide fragments of Tankyrase-II that are substantially distinct from the equivalent region of the homologous protein. Alternatively, antibodies that cross-react with Tankyrase I can be enriched by immunizing with peptide sequences that are shared between the two proteins. This is illustrated in Example 11.

Production of monoclonal antibodies is described in such standard references as Harrow & Lane (1988), U.S. Patent Nos. 4,491,632, 4,472,500 and 4,444,887, and Methods in Enzymology 73B:3 (1981). Briefly, a mammal is immunized as described above, and antibody-producing cells (usually splenocytes) are harvested. Cells are immortalized, for example, by fusion with a non-producing myeloma, transfecting with Epstein Barr Virus, or transforming with oncogenic DNA. The treated cells are cloned and cultured, and the clones are selected that produce antibody of the desired specificity.

Other methods of obtaining specific antibody molecules (optimally in the form of single-chain variable regions) involve contacting a library of immunocompetent cells or viral particles with the target antigen, and growing out positively selected clones. Immunocompetent phage can be constructed to express immunoglobulin variable region segments on their surface. See Marks et al., New Eng. J. Med. 335:730, 1996,

International Patent Applications WO 94/13804, WO 92/01047, WO 90/02809, and McGuiness et al., Nature Biotechnol. 14:1449, 1996.

Antibodies can be raised that distinguish between Tankyrase II and Tankyrase I by selecting an immunogenic peptide from a region unshared by the ANK, SAM, or PARP domains of Tankyrase I, or other proteins having one of these domains. Suitable subregions of Tankyrase II are shown in Table 3.

TABLE 3: Immunogen Sequences for Tankyrase II Specific Antibody

Amino Acid Sequence	Location	SEQ. ID NO:
MSGRRCAGGGAACASAAAEAVE	Beyond N-terminal of ANK domain (GC region)	21
TAAMPPSALPSCYKPQVLNGVRSPG ATADALSSGPSSPSSLSAASSLDNLS GSFSELSSVVSSSGTEGASSLEKKEV PGVDFSITQFVRN	Sequence between ANK and SAM domains	22
RPEGMVDG	Beyond C-terminal of PARP domain	23

Antibody molecules in a polyclonal antiserum against intact Tankyrase II can be screened to map immunogenic portions of the amino acid sequence. Sequential peptides about 12 residues long are synthesized that cover the entire protein (SEQ. ID NO:6), and overlapping by about 8 residues. The peptides can be prepared on a nylon membrane support by standard F-Moc chemistry, using a SPOTS™ kit from Genosys according to manufacturer's directions. Prepared membranes are overlaid with the antiserum, washed, and overlaid with β-galactosidase conjugated anti-immunoglobulin. Positive staining identifies antigenic regions, which, in an appropriate context, may themselves be immunogenic. There will also be antibodies that span different parts of the primary structure, or which rely on a conformational component not displayed in smaller peptides.

The antibodies of this invention can be used in immunoassays to detect or quantitate any of the polypeptides of this invention, including the natural form of Tankyrase II present in biological fluid or tissue samples. For example, it may be desirable to measure Tankyrase II in a clinical sample to determine whether the level of Tankyrase II expression is abnormal, and then correlating the finding with the presence or status of a disease associated with increased or decreased Tankyrase II activity or abundance.

General techniques of immunoassay can be found in "The Immunoassay Handbook", Stockton Press NY, 1994; and "Methods of Immunological Analysis", Weinheim: VCH Verlags gesellschaft mbH, 1993). The antibody is combined with a test sample under conditions where the antibody will bind specifically to any modulator that might be present, but not any other proteins liable to be in the sample. The complex formed can be measured in situ (U.S. Patent Nos. 4,208,479 and 4,708,929), or by physically separating it from unreacted reagents (U.S. Patent No. 3,646,346). Separation assays typically involve labeled Tankyrase-II reagent (competition assay), or labeled antibody (sandwich assay) to facilitate detection and quantitation of the complex. Assays of this nature can also be used in a competitive format to identify antibodies that bind to the same epitope on a target compound. In one such format, the reference antibody is labeled, and tested for binding to Tankyrase II in competition with a test antibody. Antibodies can also be screened to identify those with inhibitory capacity for the binding and catalytic activities of Tankyrase II.

Modulating Tankyrase II activity

This invention provides a number of different approaches to modulate Tankyrase II activity in a live cell. In one embodiment, the cell is genetically altered using a polynucleotide that affects expression of Tankyrase II at the transcription or translation level. Suitable polynucleotides include antisense sequences, ribozymes, or polynucleotides that form triplexes with the chromosomal gene for Tankyrase II, all of which were described in more detail earlier in this disclosure. In another embodiment, activity of Tankyrase II within the cell is inhibited by a peptide inside the cell that prevents Tankyrase II from exercising its usual function. Suitable peptides include intracellular antibody constructs that bind to regions of Tankyrase II necessary for catalytic or molecular binding activity, and dominant negative homologs that compete for the binding between Tankyrase II and a Tankyrase II binding partner. Proteins of this nature can be introduced into a cell by contacting the cell with a polynucleotide expression vector for the intracellular antibody or the mutant homolog.

Also contemplated are small molecule drugs that have the capability of modulating either Tankyrase II catalytic activity, or with its binding to conjugate partners. The ability to inhibit association between Tankyrase II and accessory proteins can be determined by introducing candidate inhibitors into any of the peptide binding assays described earlier, and correlating a decrease in protein complex formation with inhibitory capacity of the candidate.

Compounds can be screened for an ability to modulate ribosylation by preparing a reaction mixture comprising the test compound, either Tankyrase II or the Tankyrase II PARP domain (or a functional equivalent), the NAD^+ substrate, and a ribosylation target (Tankyrase II itself, or a Tankyrase II associated protein). Ribosylation is monitored by incorporation of ^{32}P or ^{33}P from labeled NAD^+ substrate into the solid phase, by a change of size of the target protein, or by any of the other techniques described earlier. An increase in ribosylation of the target correlates with an ability of the compound to enhance Tankyrase II ribosylation activity, while a decrease in ribosylation of the target correlates with inhibitory capacity of the test compound. The compound can also be screened in one or more parallel assays to determine whether it has the capacity to modulate the ribosylation activity of other enzymes — such as Tankyrase I, and other proteins containing PARP domains (reviewed recently by Still et al., Genomics 62:533, 1999).

Compounds that modulate the activity of Tankyrase II but not other ribosylation enzymes can be selected when it is desirable to obtain compounds that are specific for Tankyrase II. These assays can be used to screen random combinatorial libraries of small molecule compounds, or as part of rational drug design, based on known PARP inhibitors such as 3-amino-benzamide. Other potential Tankyrase II inhibitors include 4-amino-1,8-naphthalimide (Schlicker et al., Int. J. Radiat. Biol. 75:91, 1999), thiophenecaroxamides (Shinkwin et al., Bioorg. Med. Chem. 7:297, 1999), and 2-nitroimidazol-5-ylmethyl (Bioorg. Med. Chem. Lett. 9:2031, 1999).

It is potentially beneficial to modulate Tankyrase II activity in conditions associated with overexpression or underexpression of Tankyrase II. Peptides, expression systems, and small molecule drugs can also be screened according to the effect on cell biology. Cells expressing Tankyrase II treated with the test system can thereafter be monitored for an effect on telomere length as described earlier, or on replicative capacity in proliferation culture.

The following examples are provided as further non-limiting illustrations of particular embodiments of the invention.

EXAMPLES

Example 1: Identification of expressed sequence tags for Tankyrase II.

A BLAST search against the GenBank dbEST database using the Tankyrase I sequence identified several expressed sequence tags (ESTs). Many of the ESTs were identical in DNA sequence to the Tankyrase I gene. However, several ESTs coded peptides distinct from Tankyrase I. Further evaluation of these ESTs revealed they represented a distinct gene, termed Tankyrase II, since the DNA sequence identity to Tankyrase I was significantly lower than the amino acid identity, with a preponderance of silent third position codon changes.

The ESTs R64714, AA244138, and AA244137 contain sequences of the ankyrin domain of Tankyrase II; the EST AA307492 contain sequences of the SAM domain; ESTs H11865, H17748, N57467, R06946, AI247608, and R06902 contain sequences of the PARP domain. Additional 5' sequence of the 3' EST AI247608 clone revealed it diverges from the tankyrase gene and does not overlap with the SAM domain. It may contain an unprocessed intron. The 3' ESTs H11505, H17635, and N29528 were identified in GenBank as partners for the 5' ESTs H11865, H17748, and N57467, respectively. These 9 ESTs along with additional sequence obtained from the H11865, H17748, N57467, R06946 clones formed a contig containing the PARP domain and ~ 1 kbp of the 3' UTR, including a poly-A tail. These ESTs formed 3 contigs that contained 3 of the Tankyrase II domains and approximately 40 % of the coding region.

Additionally, AA088990 and AI426537 are ESTs containing the ankyrin and PARP domains of a putative mouse Tankyrase II, respectively.

Example 2: Cloning of the N-terminus of Tankyrase II.

To extend the ANK EST contig, Rapid Amplication of cDNA Ends (RACE™) (Gibco-BRL, # 18374-058) was performed using the primers tankII-2 and tankII-3 and (i) poly A+ RNA from BJ fibroblasts transduced with a retroviral vector expressing the human TERT gene (pBABE-TERT) and (ii) the Marathon-Ready testis cDNA (Clontech, #7414-1). This was followed by nested amplification using primers LtankII-1 and LtankII-2, respectively. The products from these amplifications were cloned into pCR2.1-TOPO (InVitrogen, # 45-0641). Four clones were identified by PCR with primers (AAP [Gibco] or AP-1 [Clonetech]) to the vector and the ANK EST contig (LtankII-1 or LtankII-2), termed inside-out PCR, to contain additional DNA 5' to the EST contig. Custom primers were designed based on the evolving sequence data. Subsequent sequence analysis indicated that only two, designated MP9 and MP12, contained authentic Tankyrase II sequences. "Inside-out" PCR is the term used to describe amplification using a primer pair in which one primer is from the target gene (e.g., Tankyrase II) and the second primer is specific for the vector.

Additional N-terminally extended clones were isolated by the GeneTrapper (Gibco-BRL, #10358-020) cDNA clone enrichment procedure using the oligonucleotides LtankII-4B, LtankII-5B, and LtankII-6B and plasmid cDNA libraries from liver and spleen (Gibco-BRL, #10422-012 and 10425-015, respectively). Approximately 100 GeneTrapper clones were screened by colony hybridization with a PCR probe (described *infra*) from the ANK clone AA244138 and by inside-out PCR (primers: SP6/tankII-2 and SP6/tankII-3) to identify four clones (S10, S25, S34, and L11) that contained additional DNA 5' to the EST contig.

Sequence analysis of the 2 RACE and 4 GeneTrapper clones formed a contig that extended approximately 200 bp downstream and approximately 1100 bp upstream of the original ANK EST contig. The 5' most sequence terminated in DNA homologous to the most N-terminal tankyrase ANK repeat just after the HPS domain.

Example 3: Identification of λ bacteriophage clones of Tankyrase II.

Three λ bacteriophage human cDNA libraries, λgt10 thymus (Clontech, HL1074a), λgt11 293 human embryonic kidney cancer cell line, and λTriplx Testis (Clontech, cat # HL5033t), were screened by plaque hybridization with a probe from the ANK clone AA244138. The probe was generated by PCR using the primers UtankII-5 and LtankII-7. Twenty-six phage were positively identified through secondary and tertiary plaque hybridizations. Using PCR the presence of the ANK EST contig was confirmed in all 26 phage. Additional PCR was used to identify one phage (λ11L-1-1) from the 293 library that contained the most N-terminal ankyrin repeat and the ANK, SAM, and PARP contigs. The λ11L-1-1 insert is believed to contain the entire Tankyrase II coding sequence. Two other phage λ11-L-1-3, λ11L-1-4 (293) from the 293 library were identified that contained the most N-terminal ankyrin repeats and the original ANK contig. Inside-out PCR (primers gt10-5'/LtankII-31 or gt11-5'/LtankII-31), showed these clones contained up to 800 bp of additional Tankyrase II sequence upstream of the most N-terminal ankyrin repeat. These vector/N-terminal insert PCR products and PCR products from these four phage that linked the SAM and ANK contigs and the SAM and PARP contigs were sequenced directly.

The λ11L-1-1, λ11L-1-3, or λ11L-1-4 can be individually characterized by the following tests. Phage λ11L-1-1 contains DNA that can be amplified with the primer pairs UtankII-5/LtankII-16, UtankII-5/LtankII-9, and UtankII-3/LtankII-10 (Table 4). Phage λ11L-1-3 contains DNA that can be amplified with the primer pair UtankII-5/LtankII-16, but not with the primer pairs UtankII-5/LtankII-9 and UtankII-3/LtankII-10 (Table 4). Phage λ11L-1-4 contains DNA that can be amplified with the primer pairs UtankII-5/LtankII-16, UtankII-5/LtankII-9, but not with the primer pair UtankII-3/LtankII-10 (Table 4). Additional sequence was obtained by amplifying phage λ11L-1-4 DNA using UTankII-3 and LTankII-11primers (PARP/SAM spanning sequence).

Example 4: Tankyrase II amino-terminal domain sequence

More than 200 GeneTrapper clones obtained using UTankII-4B, UTankII-5B and UTankII-6B oligonucleotides were probed by colony hybridization with a ^{32}P-labeled PCR fragment from clone MP9 (*supra*) using primers UTank2-30 and LTank2-1. Of the positive clones identified, sequence was obtained from 5 independent clones from two different cDNA libraries (SuperScript human liver cDNA and SuperScript human spleen cDNA libraries [Clonetech]). The clones are: S4.66, S4.21, S6.7, S6.91, L5.4. Of these L5.4 had the longest 5' sequence. L5.4 was deposited with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, VA) and assigned Accession No. 203919. The DNA encoding the amino-terminal region of the Tankyrase II polypeptide is extremely GC-rich (>80% in the sequence).

Figures 2 and 3 show cDNA and protein sequence data obtained for Tankyrase II.

TABLE 4: Primers for amplifying Tankyrase II sequences

Forward & Reverse Primer Designation	Nucleotide Sequence	SEQ. ID NO:
UTANKII-1	GTT ACA TTT GCC ACA GGC AG	24
UTANKII-2	GTC TTT CTT GCA GTT CAG TG	25
UTANKII-3	GAG TCG AGA GAC TTA TCT CC	26
UTANKII-4A	GAG CAC AGA GAT GGA GGT C	27
UTANKII-4B	ATG TAC AGC AAC TCC TCC AAG A	28

TABLE 4: Primers for amplifying Tankyrase II sequences

Forward & Reverse Primer Designation	Nucleotide Sequence	SEQ. ID NO:
UTANKII-5A	CAG ACA ATT GCT GGA AGC TG	29
UTANKII-5B	CAG ACA ATT GCT GGA AGC TGC A	30
UTANKII-6A	CTA CTC CTG AGC TAT GGG TG	31
UTANKII-6B	GTG TAC TGT TCA GAG TGT CAA C	32
LTANKII-7	CCA TGC TGG AGC AGA AGT TTG	33
LTANKII-8	GCT AAA ATC TCT CCT GGA ACC	34
LTANKII-9	GTT TGT GCC TAT GTC CAT AAG C	35
LTANKII-10	CAA AAG AGC AGC TGC CTG TG	36
LTANKII-11	CTG CAG GAA AGA CTT TCC CAA G	37
UTANKII-12	GCA GCC AGT GGC CCT CTA CG	38
UTANKII-13	GCC CCA CAG GCC TGT GGC C	39
UTANKII-14	GAA ACT AAT TCC CAC TAA CC	40
LTANKII-15	AAT AAA TAC TGG GCT AGT AC	41
LTANKII-16	AGG GTC TGC ACC ATG CTG GAG C	42
LTANKII-17	ATA AAT CAG CTA CAT TAA CTA C	43
LTANKII-18	CCC AGC TGC AAA ATG AAG T	44
LTANKII-19	AAT GAC TCT GCA GTT GAC AC	45
UTANKII-20	GAT ACA CTC ACC GGA GAA AAG	46
LTANKII-21	GTG AAC TGG ACA CCC AGT ACC	47
UTANKII-22	GGT ATG GTC GAT GGA TAA ATA G	48
LTANKII-23	GAA CAC AGT ATT GTA TTA G	49
UTANK2-30	CGG CGG GCA GGA AAT CCA CC	50
LTANK2-31	TTG GGG TCT GCA CCA TGT CG	51
UTANK2-32	TCC AGA GGC TGG TGA CCC CTG A	52
LTANKII-33	TCT GCT AAA TCC AAT GCT GTC C	53
LTANKII-34	TGC AGC GGG GTG GAT TTC CT	54
LTANKII-35	CAT TTT GAA GCA AAT ATT TA	55
LTANKII-36	GGA ATA AGG CCC CCA TTA TA	56
LTANKII-35	CAT TTT GAA GCA AAT ATT TA	57
LTANKII-36	GGA ATA AGG CCC CCA TTA TA	58

Example 5: Northern hybridization of Tankyrase II mRNA

A Northern blot (Human Multiple Tissue Northern (MTN) TM Blot™ (obtained from Clontech, Cat #7780-1) was hybridized with a 3'UTR probe at 2 x 10^6 cpm/ml hybridization solution. The 3' UTR fragment was amplified by PCR with UTank2-14 and LTank2-15 primers using the Est clone n57467 as a template.

The Northern analysis showed that the Tankyrase II transcript is about 6 to 7.5 kb in length, and is expressed in most tissues, including brain, heart, colon, thymus, spleen, kidney, liver, small intestine, lung, and

peripheral blood leukocytes. It appears to be particularly abundant in skeletal muscle and placenta. "BJ RNA" is polyadenylated RNA isolated from a human fibroblast cell line designated BJ.

Example 6: Plasmid clones of the Tankyrase II cDNA

The isolated Tankyrase II cDNA bacteriophage clones were transferred to plasmid vectors as follows:

1. The Tankyrase II cDNA contained in bacteriophage λ11L-1 was removed as a BsiW1 fragment and inserted into the Acc65 I site of pBluescript II SK+ (Stratagene) (designated pGRN509).
2. The Tankyrase II cDNA contained in bacteriophage λ11L-3 was removed as a BsiW1 fragment and inserted into the Acc65 I site of pBluescript II SK+ (Stratagene) (pGRN510).
3. The Tankyrase II cDNA contained in bacteriophage λ11L-4 was removed as a BsiW1 fragment and inserted into the Acc65 I site of pBluescript II SK+ (Stratagene) (pGRN511).

The PARP domain from pGRN509 was amplified with the primer hParp1 (5'-CC ATCGAT GCCAGCCATG GAG GTT CCA GGA GTA GAT-3'; SEQ. ID NO:59) and primer hParp2 (5'- GCTCT AGA TCA GGC CTC ATA ATC TGG-3'; SEQ. ID NO:60) using PFU/Taq polymerase mixture. The resulting fragment was TA cloned into the InVitrogen TA cloning vector pCR2.1-TOPO®. A clone (pGRN513) was selected with the sense strand downstream of the T7 promoter. The primer hParp1 introduces an ATG and kozak consensus sequence at the 5' end of the PARP

To assemble a full length cDNA containing the Tankyrase II ORF fragments from pGRN511 and pGRN509 were combined as follows: The Not I fragment of pGRN511 was inserted into the Not I site of pBluescript II KS+ (Stratagene) (pGRN512). pGRN512 was digested with Nhe I and Cla I and the larger vector/Tankyrase II cDNA fragment was isolated, the ~2.1 Kbp Nhe I - Cla I fragment of pGRN509 was ligated to this fragment to generate a clone containing the full length Tankyrase II cDNA ORF (pGRN514).

Example 7: Further Sequence Data for the Tankyrase II cDNA

The plasmids pGRN509 and pGRN511 were sequenced with an ABI 377 automated DNA sequencer by standard techniques using primers complementary to the insert sequences.

Figure 4 shows the revised cDNA sequence (SEQ. ID NO:5), and the revised amino acid translation (SEQ. ID NO:6). The translated protein product is presumed to begin at the Met encoded at position 224 of the cDNA sequence, and ending at position 3721. The Met is assigned position No. 1 for purposes of numbering the amino acid translation. However, the upstream polynucleotide sequence shown contains no stop codon, and the translation starting Met may be further upstream from the insert shown in the figure.

The number of amino acids in Tankyrase II corresponding to nucleotides 284 to 3721 is 1166 amino acids long. The calculated molecular weight is 126.8 kDa, and the calculated isoelectric point is 6.78.

Figure 1 shows the location of the functional domains in the Tankyrase II sequence. The position of each domain within the sequence is shown in Table 5.

TABLE 5: Location of Functional Domains in Tankyrase II

Domain	Position
GC	1 to 22
ANK	23 to 859
SAM	870 to 935
PARP	1023 to 1161

Figure 5 and Figure 6 compare Tankyrase II (SEQ. ID NO:6) with its closest known intraspecies homolog, Tankyrase I (SEQ. ID NOs:8), at the protein level, and at the cDNA level.

The degree of sequence identity of Tankyrase II relative Tankyrase I was determined in this example by dividing the two proteins into their functional domains. Identity was then calculated by dividing the number of matched residues by the number of matched and mismatched residues over each area, scoring half a point for each unmatched residue occurring in a gap or overhang on either side. Results were as follows:

- N-terminus: 7 matches/ 7 matches + 20 mismatches + (79 gaps/2) = 7%
- Ankyrin repeats: 720/ 720+104+(7/2) = 87%
- Inter domain 1: 7/7+6+(2/2)= 50%
- SAM domain: 54/54+12= 82%
- Inter domain #2: 82/82+15= 83%
- PARP domain: 132/132+7=95%
- C-terminus: 0/5 +(8/2)= 0%
- Overall: 992/1241.5= 79.9%
- Overall (discounting N- and C-termini): 985/1133.5= 86.9%

Example 8: Testing for PARP Activity

To produce a Tankyrase II peptide comprising the PARP domain, pGRN513 was transcribed and translated in a 20 fold scale up in vitro coupled transcription/translation (TnT) reaction. Full-length Tankyrase II peptides can be obtained by similar procedures, using plasmids designated pGRN514 or pGRN323.

Each plasmid was set up as follows, paired with a reaction in which the plasmid DNA was omitted as an unprogrammed control. The reaction mixture contained 20 µg of circular plasmid pGRN513 or pGRN514 or pGRN523; 500 µl rabbit reticulocyte lysate; 1x TnT Buffer; 20 µl T7 RNA polymerase; 20 µl 1 mM complete amino acids; 20 µl RNAguard™; and dH_2O to 1 ml total volume. The reactions were incubated for 90 minutes at 30°C, then pooled and made 50% with ammonium sulfate. The resulting pellets were washed with 50% ammonium sulfate and resuspended in either 400µl PARP buffer A (50mM Tris-HCL pH 8, 4mM $MgCl_2$, 0.2 mM DTT, 50 mM NaCl, 10mM ß-mercaptoethanol, 1 mM PMSF) for the Tankyrase II reactions, or in 100 µl PARP buffer A for the unprogrammed control. The TnT resuspended lysate was then dialysed overnight against two changes of PARP buffer A to remove traces of ammonium sulfate.

The following assays were performed to determine PARP activity:

1. ^{35}S-labelled Tankyrase without NAD^+
2. ~150ng Tankyrase II PARP domain, with NAD^+
3. ~150ng Tankyrase II PARP domain, with NAD^+, 1.5µg TRF1, 5 µg Histones
4. ~150ng Tankyrase II PARP domain, with NAD^+, 1.5µg TRF1, 5 µg Histones
5. ~150ng Tankyrase II PARP domain, with NAD^+, 1.5µg TRF1, 5 µg Histones and 1.6 mM 3-aminobenzamide
6. Unprogrammed lysate with NAD, 1.5µg TRF1, 5µg Histones
7. PARP control enzyme (Trevigen cat#4667-50-01), with NAD^+, TRF1, Histones

For Reaction 1, Tankyrase II was biosynthetically labeled using [^{35}S]methionine as a molecular weight marker of the non-ribosylated form. The reaction mixture comprised 1 µg of circular plasmid pGRN513 or pGRN514 or pGRN523; 25 µl rabbit reticulocyte lysate; 1x TnT Buffer; 1 µl T7 RNA polymerase; 1 µl 1mM

methionine; 2 µl [^{35}S] Met (1000Ci/mmol); 1 µl RNAguard™; and dH20 to 50 µl total volume. The product was precipitated with 50% ammonium sulfate but not dialysed.

Reactions 2 to 6 were conducted under the following assay conditions: 1x PARP enzyme buffer (Trevigen Cat#4667-50-02), 40 µM [^{32}P]NAD$^+$ (50 µCi). Reactions 2,3 5-7 with TCA were precipitated in 20% TCA, pellets were washed sequentially with 5% TCA, 90% acetone/1 N HCl and 100% acetone. Pellets were then resuspended in 40 µL protein loading buffer and heated for 10 minutes at 80°C.

Reaction 4 was immunoprecipitated with 10 µL Anti-poly (ADP-ribose) monoclonal antibody (Trevigen Cat# 4335-MC-100) and 10 µL Anti-poly (ADP-ribose) polyclonal antibody (Biomol Cat# SA-276). Reaction volume was incubated on ice for 30 minutes in the presence of the antibodies. 40 µL Protein A slurry was incubated by rotating at room temperature for 2 hours. Beads were washed twice with PARP buffer A containing 50 mM NaCl, and once in PARP buffer A containing 450 mM NaCl. Beads were boiled in 40 µL protein loading buffer. Reaction 7 was performed according to manufacturer's directions (Trevigen). Samples from these reaction mixtures were then analysed on a 12% SDS-PAGE gel. The gel was dried and exposed to a phosphorimager screen and imaged. Preliminary results of these assays have been inconclusive.

Example 9: Chromosomal location of the Tankyrase II gene

The Tankyrase II gene was localized to chromosome 10q by radiation hybrid mapping (Boehnke et al., Am J. Hum Genet 49:1174, 1991; Walter et al., Nature Genet 7:22) using the medium resolution Stanford G3 panel of 83 RH clones of the whole genome (created at the Stanford Human Genome Center). A human lymphoblastoid cell line (donor; rM) was exposed to 10,000 rad of X-rays and was then fused with non-irradiated hamster recipient cells (A3). Eighty-three independent somatic cell hybrid clones were isolated, and each represents a fusion event between an irradiated donor cell and a recipient hamster cell. The panel of G3 DNA was used for ordering markers in the region of interest as well as establishing the distance between these markers.

The primers used for RH mapping were UTANKII-20 and LTANKII-21 (Table 4). The 83 pools were amplified independently and 13 (16%) scored positive for Tankyrase II. The amplification results were submitted to the Stanford RH server, which then provided the map location, 10q23.3, and the closest marker, STS D10S536.

Example 10: Transcription and Translation of Tankyrase II PARP domain

pGRN513 was tested by in vitro transcription/translation (TnT) to confirm it encoded the appropriate sized protein. The PARP domain is expected to run at approximately 35 kDa, as determined by SDS polyacrylamide gel electrophoresis. TnT reactions were set up for pGRN513 and pGRN125 (hTERT as a positive control for the TnT reaction) with the following components:

- 1 µg of circular plasmid
- 25 µl rabbit reticulocyte lysate
- 1 x TNT Buffer (Promega Cat # L4810)
- 1 µl T7 RNA polymerase
- 1 µl 1mM methionine
- 2 µl [^{35}S] methionine (1000 Ci /mmol) (Amersham Cat # SJ 1015, 1000 Ci/mmol)
- 1 µl RNAguard (Pharmacia Cat # 27-0815-01)
- dH20 to 50 µl total volume

The reaction was incubated for 90 minutes at 30°C. 40 µl of the TnT reactions were precipitated with 50% ammonium sulfate and resuspended in 40 µl of buffer (20mM HEPES-KOH pH 7.9, 2 mM $MgCl_2$, 1mM EGTA, 10% glycerol, 0.1% Nonidet P-40, 0.1mM phenylmethylsulphonyl fluoride) /100mM NaCl. 5 µl of TnT reaction, 5 µl of ammonium sulfate cut TnT reaction and 5 µL of the ammonium sulfate cut was analyzed on a 12% SDS-PAGE. pGRN513 generated the expected size fragment.

Example 11: Antibodies to Tankyrase II

Peptides are prepared on a synthesizer for use as immunogens based on the sequence data shown in Table 6.

TABLE 6: Peptide Immunogens

Laboratory Designation	Sequence	Specificity	SEQ. ID NO:
GCJT-1	MAASRRSQC	residues 1-8 of Tankyrase I	61
GCJT-2	MSGRRCAGK	residues 1-8 of Tankyrase II	62
GCJT-3	QEGISLGNSEADRQC	residues 481-494 of Tankyrase II	63
QCJT-4	GEYKKDELLEC	residues 269-276 of Tankyrase II; common to both proteins	64

Underlined residues do not belong to the native sequence of the proteins but are added to the peptides in order to couple them to carriers for antibody production.

BIOLOGICAL DEPOSIT

Phage λ11L-1-1, λ11L-1-3, λ11L-1-4 were deposited as a mixture with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, VA) on April 12, 1999, under Accession No. 203919.

Phage are stored in a buffer of 5.8 g NaCl, 2 g $MgSO_4 \cdot \&H_2O$, 50 mL 1 M Tris-HCl pH 7.5, and 0.01% gelatin (Difco). To isolate each phage, test individual phage are separated by plaque purification by standard PCR amplification. Tankyrase II sequence in phage λ11L-1-1 can be amplified with the primer pairs UtankII-5/LtankII-16, UtankII-5/LtankII-9, and UtankII-3/LtankII-10 (Table 4, supra). Tankyrase II sequence in phage λ11L-1-3 can be amplified with the primer pair UtankII-5/LtankII-16, but not with the primer pairs UtankII-5/LtankII-9 and UtankII-3/LtankII-10. Tankyrase II sequence in phage λ11L-1-4 contains DNA that can be amplified with the primer pairs UtankII-5/LtankII-16, UtankII-5/LtankII-9, but not with the primer pair UtankII-3/LtankII-10.

TABLE 7: Sequences Listed in this Disclosure

SEQ. ID NO:	Subject	Reference
1	Human Tankyrase II DNA sequence	FIGURE 2, this Invention. (60/128,577)
2	Human Tankyrase II protein sequence	FIGURE 2, this Invention. (60/128,577)
3	Human Tankyrase II DNA sequence	FIGURE 3, this Invention. (60/129,123)
4	Human Tankyrase II protein sequence	FIGURE 3, this Invention. (60/129,123)
5	Human Tankyrase II DNA sequence	FIGURE 4, this Invention.
6	Human Tankyrase II protein sequence	FIGURE 4, this Invention.
7	Human Tankyrase I DNA sequence	GenBank Accession No. AF082556 Smith et al. Science 282:1484 (1998)
8	Human Tankyrase I protein sequence	GenBank Accession No. AF082556 Smith et al. Science 282:1484 (1998)
9	Human cDNA clone similar to Ankyrin G119 mRNA	GenBank Accession No. AA244138 R. Strausberg (unpublished)
10	Human cDNA clone similar to Ankyrin-related ADP-ribose polymerase mRNA	GenBank Accession No. AW157349 L. Hillier et al. (unpublished)

TABLE 8: Additional Sequence Data

GenBank Accession No.	Subject	Reference
U40705	TRF1 sequence	Chong et al., Science 270:1663 (1995)
AF002999	TRF2 sequence	Broccoli et al., Nature Genet. 17: 231 (1997)
AF195512	TIN2 sequence	Kim et al., Nat. Genet. 23 :405 (1999)

- For purposes of prosecution in the U.S.A., the DNA and encoded amino acid sequences listed in this are hereby incorporated herein by reference.

CLAIMS

What is claimed as the invention is:

1. An isolated polynucleotide having at least one of the following properties:

a) it comprises a sequence of at least 30 consecutive nucleotides that is contained in SEQ. ID NO:5, but not in SEQ. ID NO:7;

b) it comprises a sequence of at least 30 consecutive nucleotides that is contained in plasmids deposited under Accession No. 203919, but not in λ-phage or SEQ. ID NO:7;

c) it comprises a sequence of at least 100 consecutive nucleotides that is at least 90% identical to a sequence contained in SEQ. ID NO:5, but not in SEQ. ID NO:7;

d) it comprises a sequence of at least 100 consecutive nucleotides that is at least 90% identical to a sequence contained in plasmids deposited under Accession No. 203919, but not in λ-phage or SEQ. ID NO:7; or

e) it comprises a sequence of at least 30 consecutive nucleotides that hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1, but not to a polynucleotide with the sequence in SEQ. ID NO:7.

2. The isolated polynucleotide of claim 1, which encodes a protein comprising a GC domain, a PARP domain, a SAM domain, or an ANK domain.

3. The isolated polynucleotide of claim 1 or claim 2, which encodes a protein having at least one of the following properties:

a) it binds to any of the proteins TRF1, TRF2, TIN2, and Tankyrase I; or

b) it ADP-ribosylates a target protein in the presence of nicotinamide adenine dinucleotide (NAD^+).

4. An isolated polypeptide having at least one of the following properties:

a) it comprises a sequence of at least 10 consecutive amino acids that is contained in SEQ. ID NO:6, but not in SEQ. ID NO:8;

b) it comprises a sequence of at least 10 consecutive amino acids that is contained in plasmids deposited under Accession No. 203919, but not in λ-phage or SEQ. ID NO:8;

c) it comprises a sequence of at least 25 consecutive amino acids that is at least 90% identical to a sequence encoded within SEQ. ID NO:6, but not in SEQ. ID NO:8; or

d) it comprises a sequence of at least 25 consecutive nucleotides that is at least 90% identical to a sequence encoded within plasmids deposited under Accession No. 203919, but not in λ-phage or SEQ. ID NO:8.

5. The isolated polypeptide of claim 5, comprising a GC domain, a PARP domain, a SAM domain, or an ANK domain.

6. The isolated polypeptide of claim 4 or claim 5, having at least one of the following properties:

a) it binds to any of the proteins TRF1, TRF2, TIN2, and Tankyrase I; or

b) it ADP-ribosylates a target protein in the presence of nicotinamide adenine dinucleotide (NAD^+).

7. Isolated human Tankyrase II protein, at least 10-fold more pure on a weight per weight basis than occurs in natural sources.

8. An isolated polynucleotide encoding the polypeptide of any of claims 4-7.

9. An isolated polyclonal antibody or a monoclonal antibody that binds specifically to a polypeptide with the sequence SEQ. ID NO:6 but not to a polypeptide with the sequence in SEQ. ID NO: 8.

10. The antibody of claim 9, which inhibits the catalytic activity of Tankyrase II; inhibits the binding of Tankyrase II to any of the proteins TRF1, TRF2, TIN2, or Tankyrase I; or inhibits protein ribosylation mediated by Tankyrase II in the presence of NAD^+.

11. A method for obtaining an antibody according to claim 9 or 10, comprising immunizing an animal or contacting an immunocompetent particle with a polypeptide according to any of claims 4-7.

12. A method for isolating a peptide according to any of claims 4-7 from a protein mixture, comprising incubating the mixture with an antibody according to claim 9 or 10 under conditions that permit specific binding of the peptide to the antibody to form a complex, separating antibody-peptide complexes from the mixture, and recovering the peptide from such complexes.

13. A method for obtaining a peptide according to any of claims 4-7, comprising expressing a polynucleotide of any of claims 1-3 and claim 8 in a host cell.

14. A method for ribosylating a target protein, comprising incubating the target protein with a peptide according to any of claims 4-7 in the presence of NAD^+.

15. An assay method for determining Tankyrase II binding activity in a sample, comprising incubating a sample suspected of containing a protein having Tankyrase II binding activity with a peptide according to any of claims 4-7 under conditions where the protein can bind the peptide specifically to form a complex, and then correlating any complex formed with the presence or amount of the protein in the sample.

16. The assay method of claim 15, wherein the protein that has Tankyrase II binding activity is selected from the group consisting of TRF1, TRF2, TIN2, and Tankyrase I.

17. A method of screening a test compound for an ability to affect Tankyrase II activity, comprising incubating a reaction mixture containing a peptide according to any of claims 4-7, a protein that has Tankyrase II binding activity, and the test compound under conditions where the protein could bind the peptide to form a complex in the absence of the test compound; and determining any effect of the test compound on the amount or rate of complex formation.

18. A method of screening a test compound for an ability to affect Tankyrase II activity, comprising incubating a reaction mixture containing a peptide according to any of claims 4-7, a target protein, nicotinamide adenine dinucleotide (NAD^+), and the test compound, under conditions where the target protein would be ribosylated by the peptide in the absence of the test compound; and determining any effect of the test compound on the amount or rate of ribosylation.

19. A method for modulating Tankyrase II expression in a cell, comprising contacting the cell with the polynucleotide of any of claims 1-3 which is an antisense polynucleotide, a ribozyme, or interfering RNA (RNAi) under conditions where the polynucleotide inhibits translation of mRNA into Tankyrase II.

20. A method for modulating telomere length in a cell, comprising modulating the expression of Tankyrase II in the cell.

Figure 1

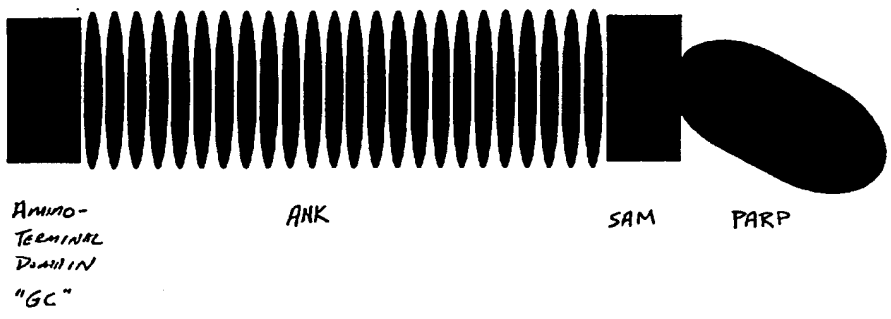
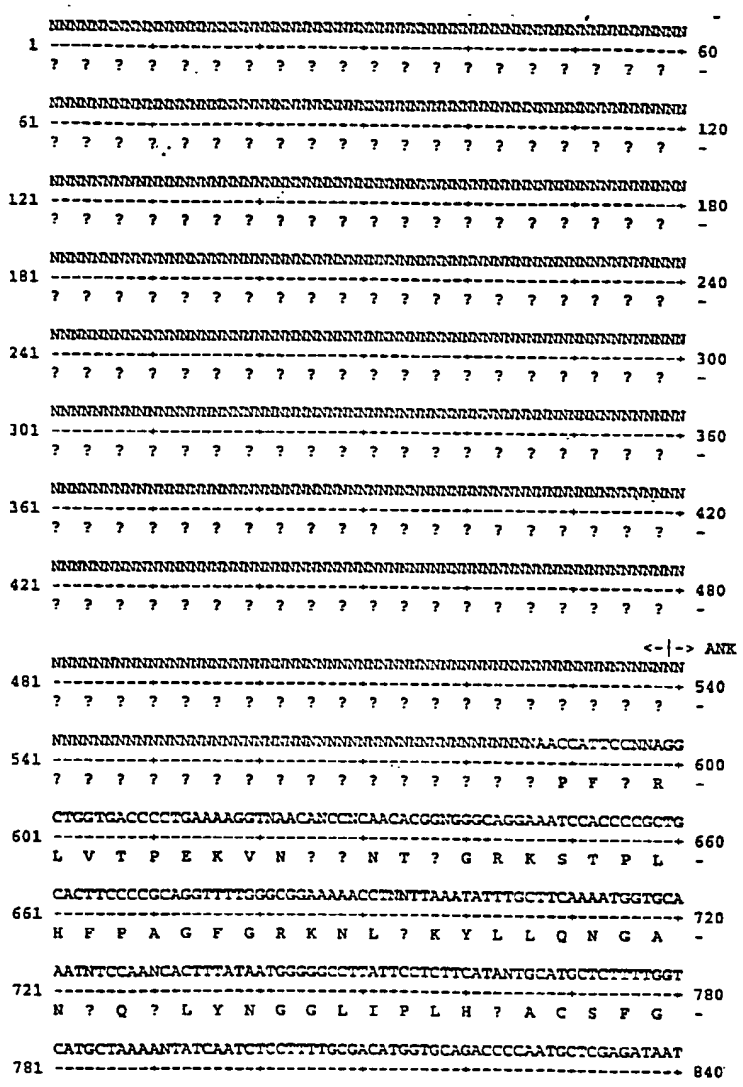


Figure 2(1)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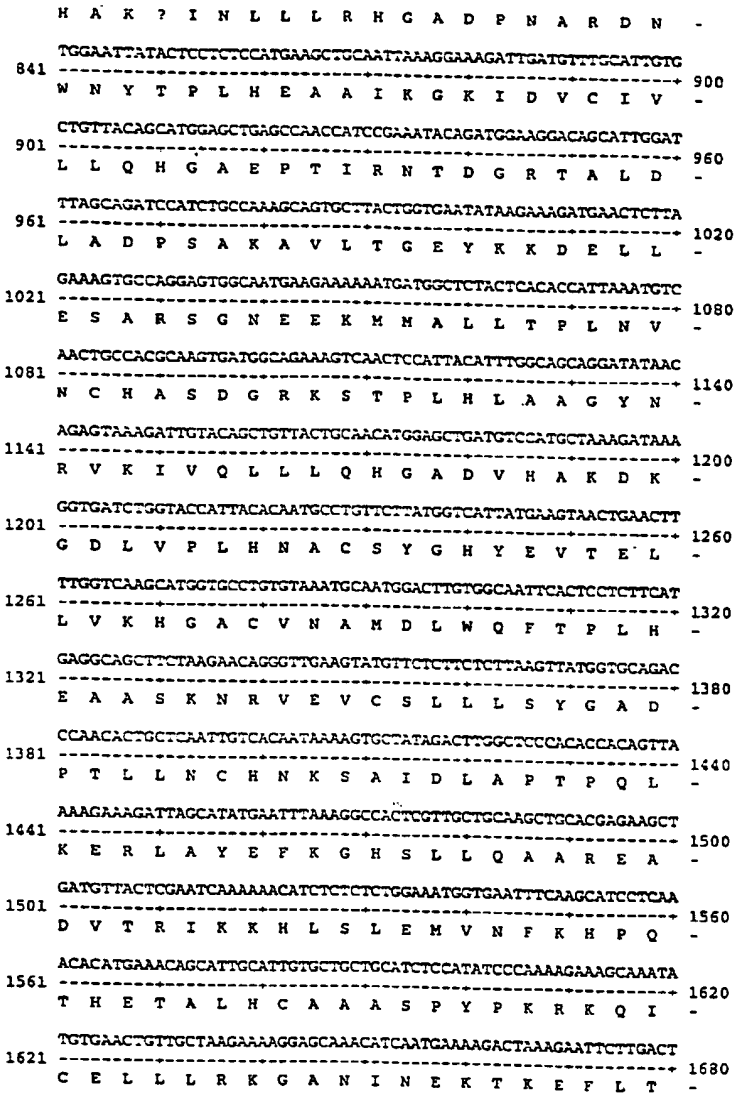
840

Figure 2(2)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1021
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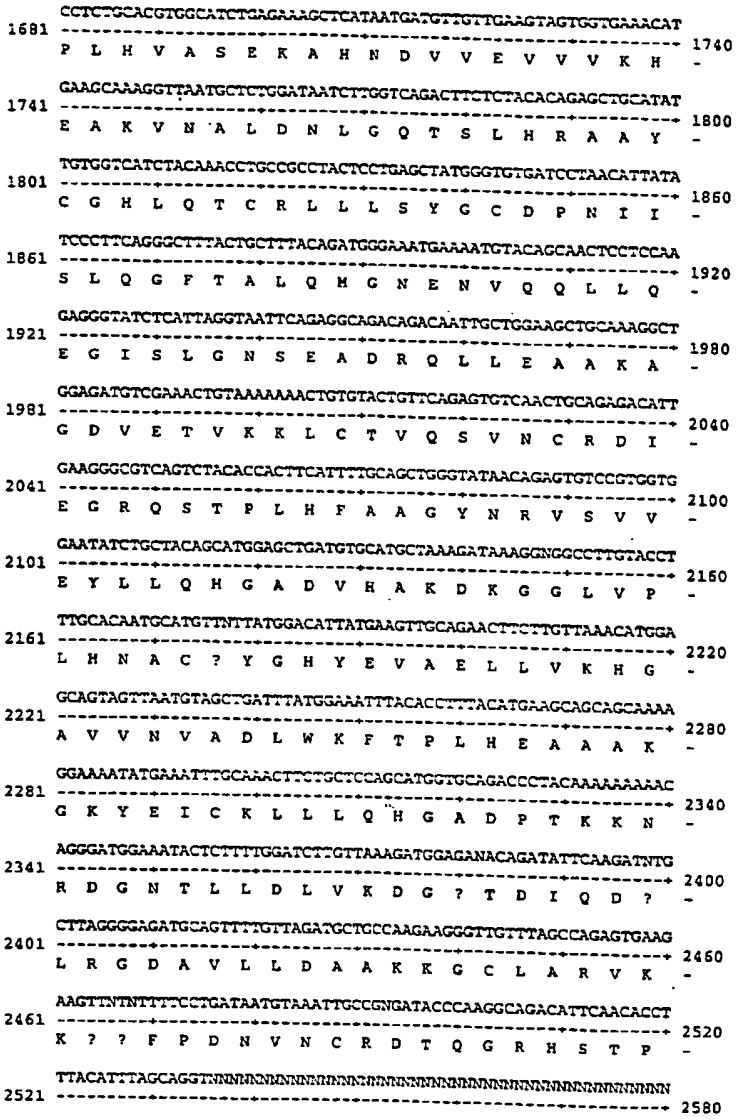
Figure 2(3)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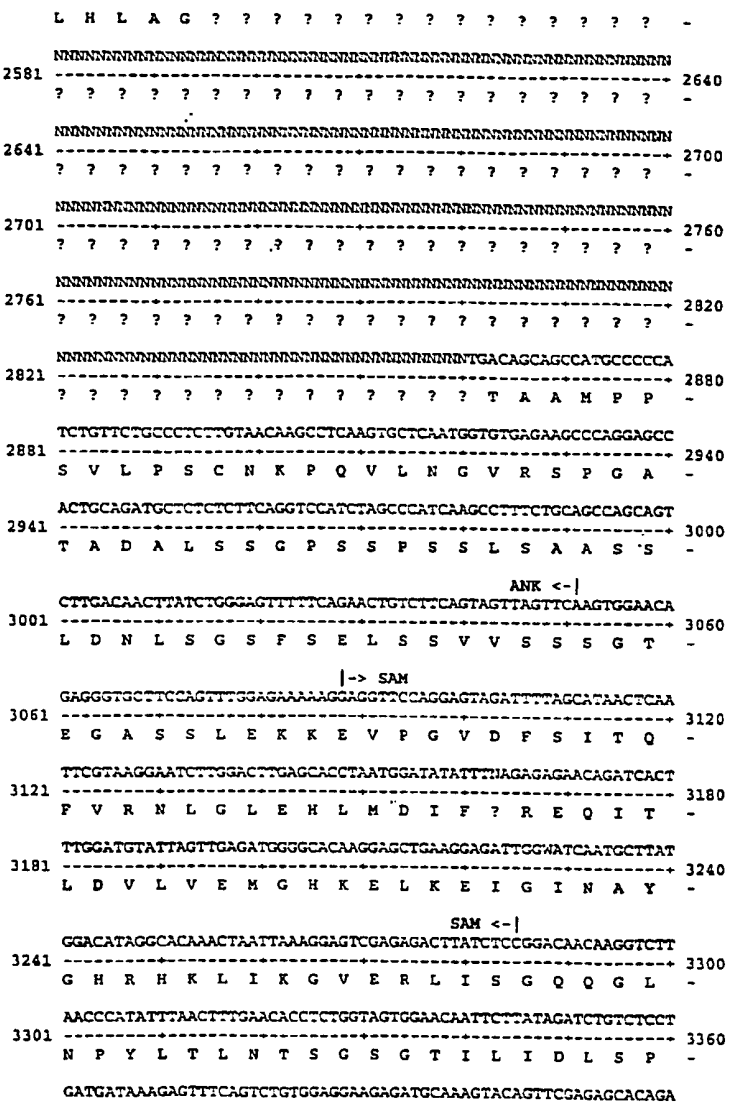

Figure 2(5)

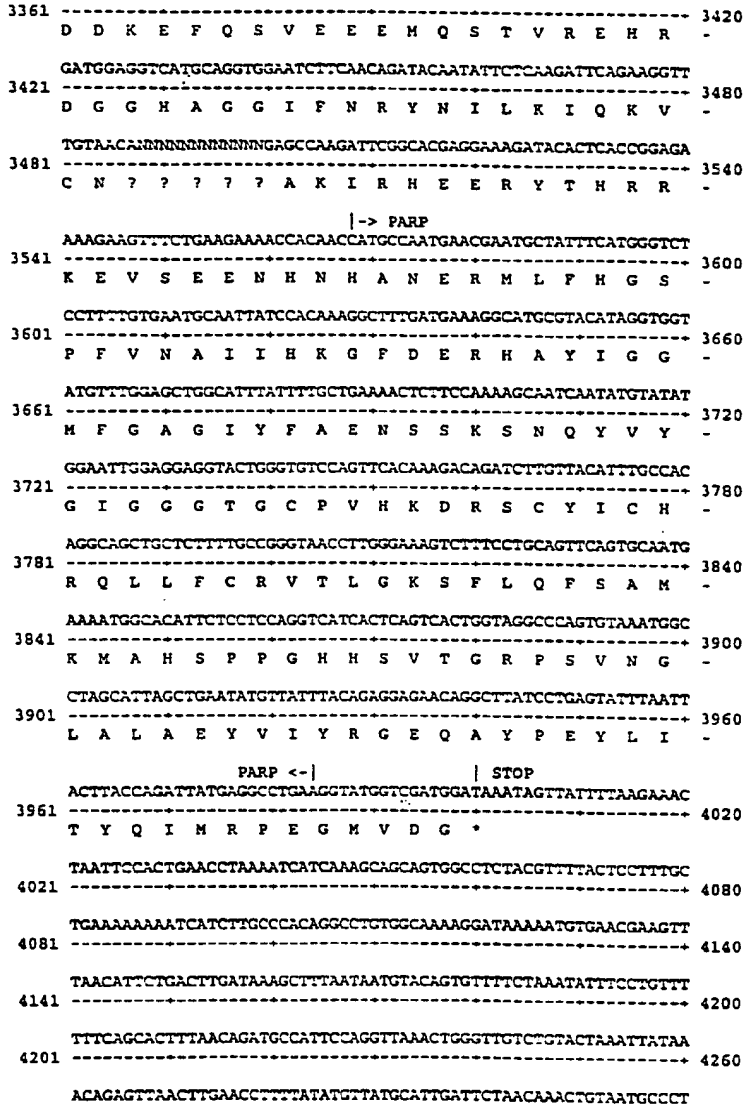
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Figure 2(6)

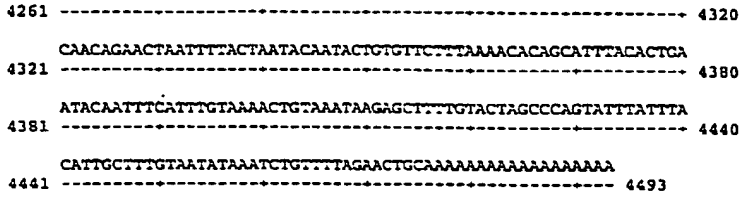
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Figure 3(1)

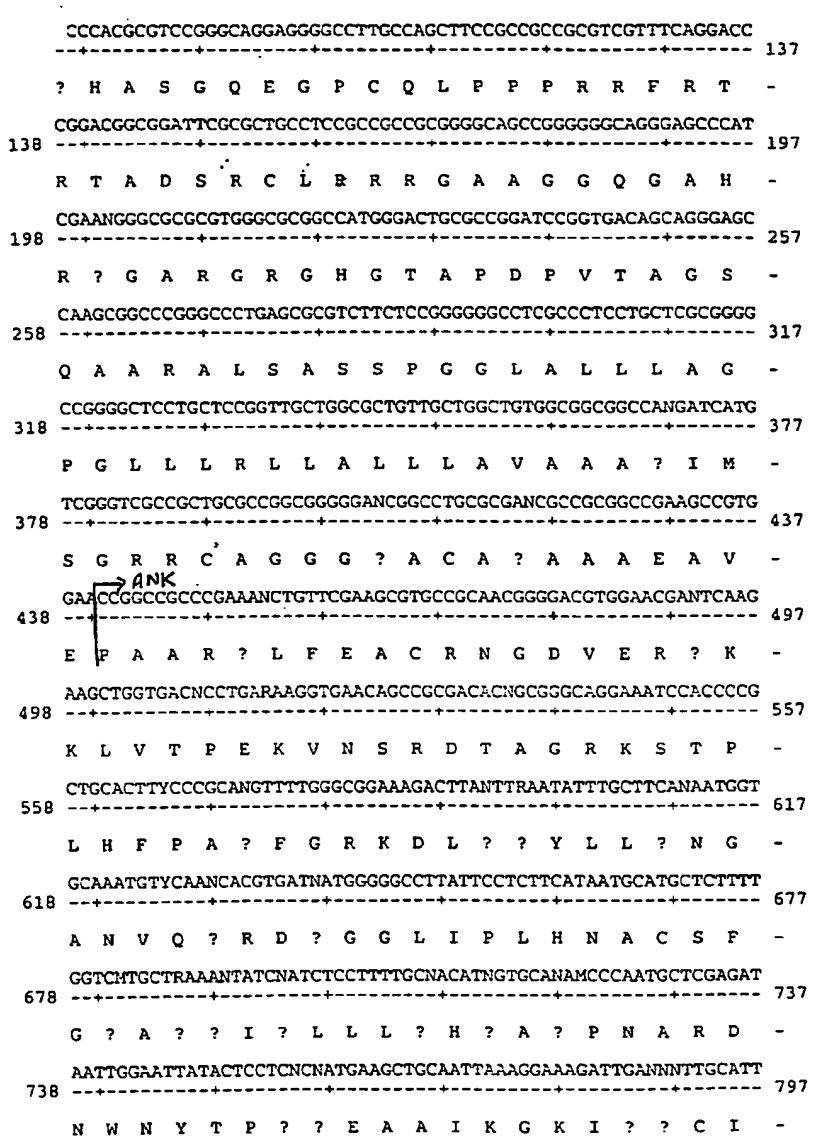
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Figure 3(2)

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Figure 3(3)

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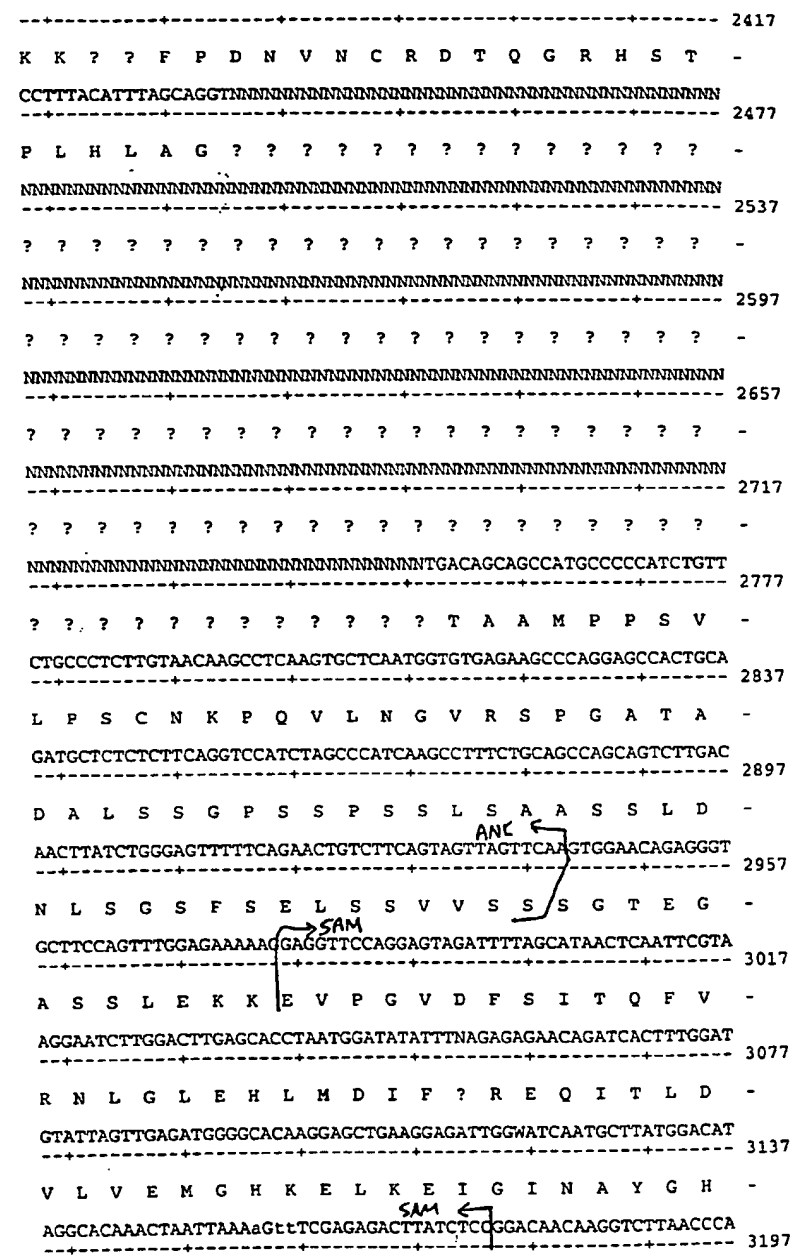
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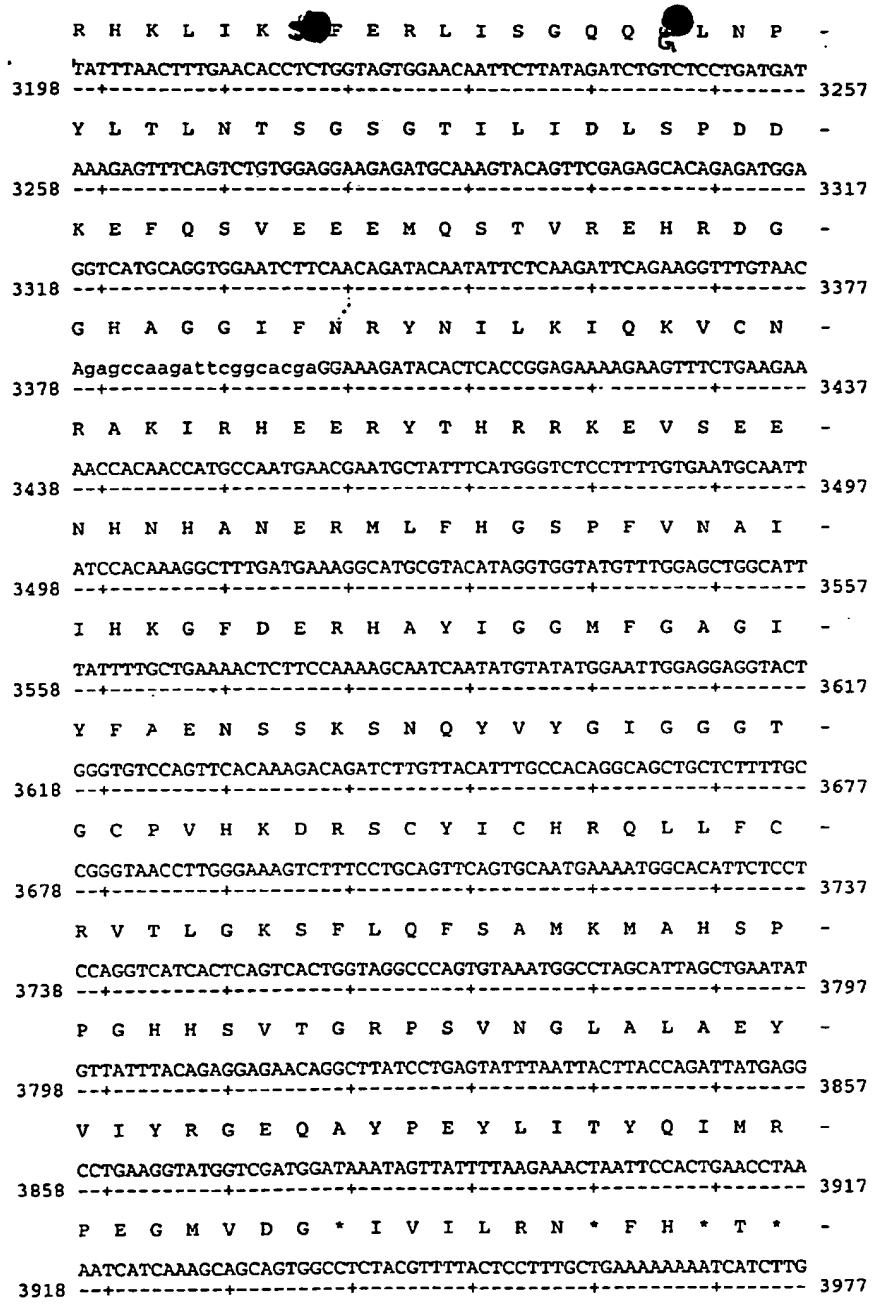
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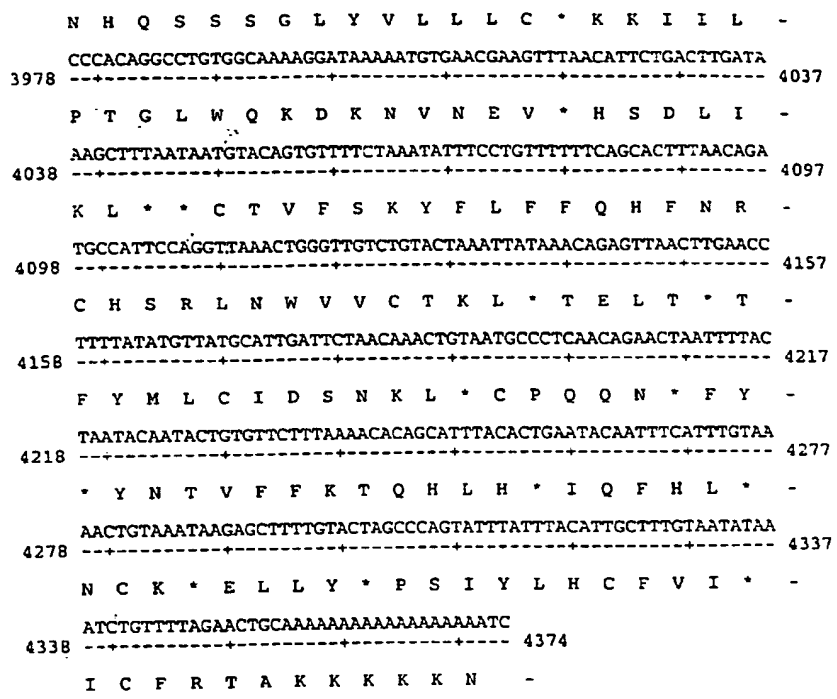
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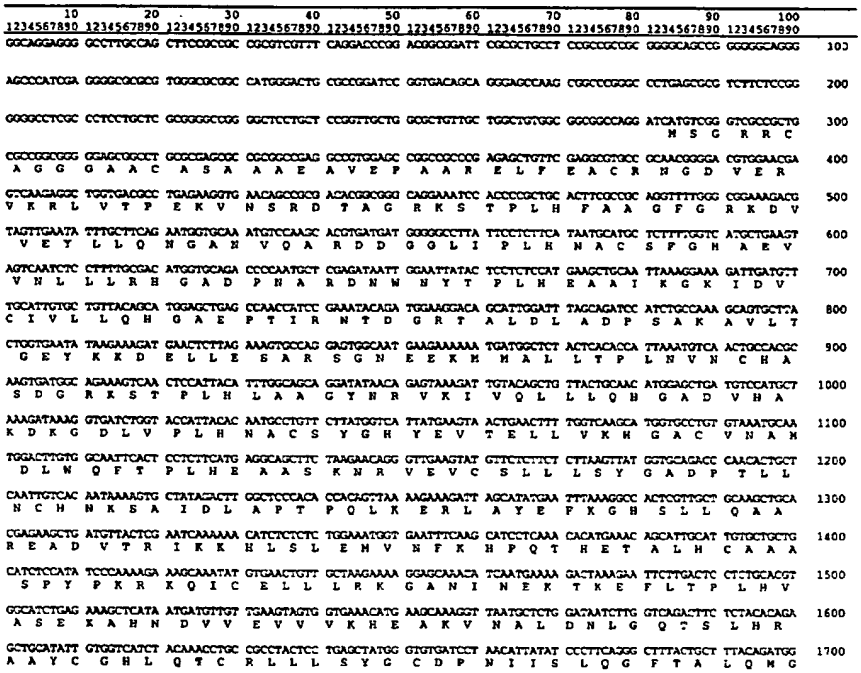

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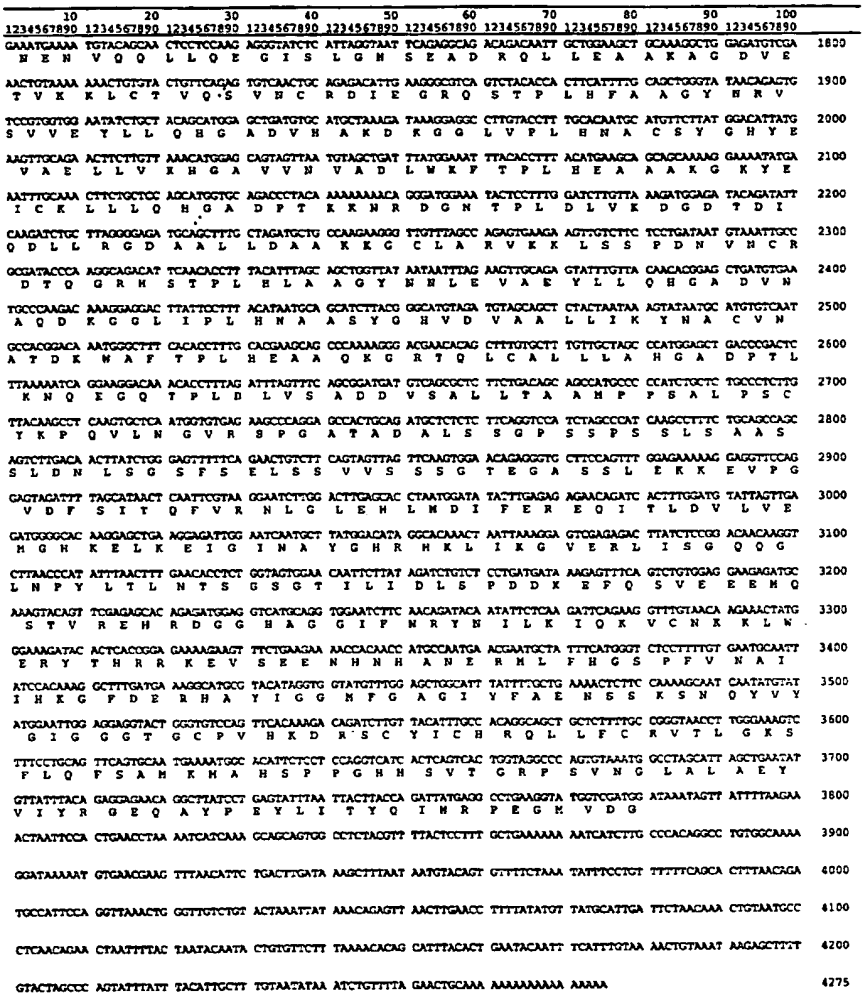

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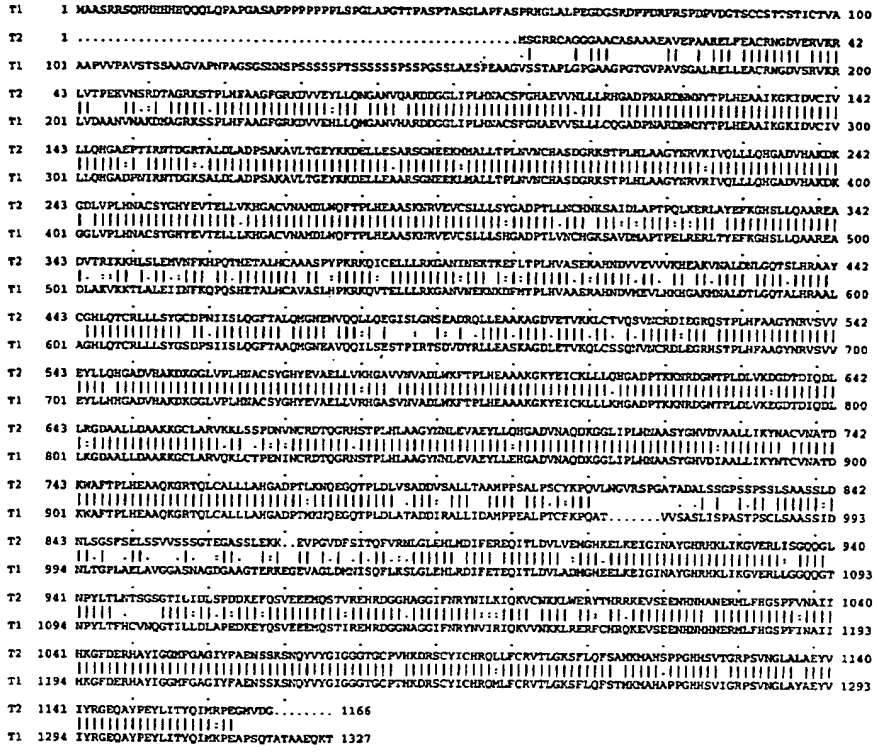

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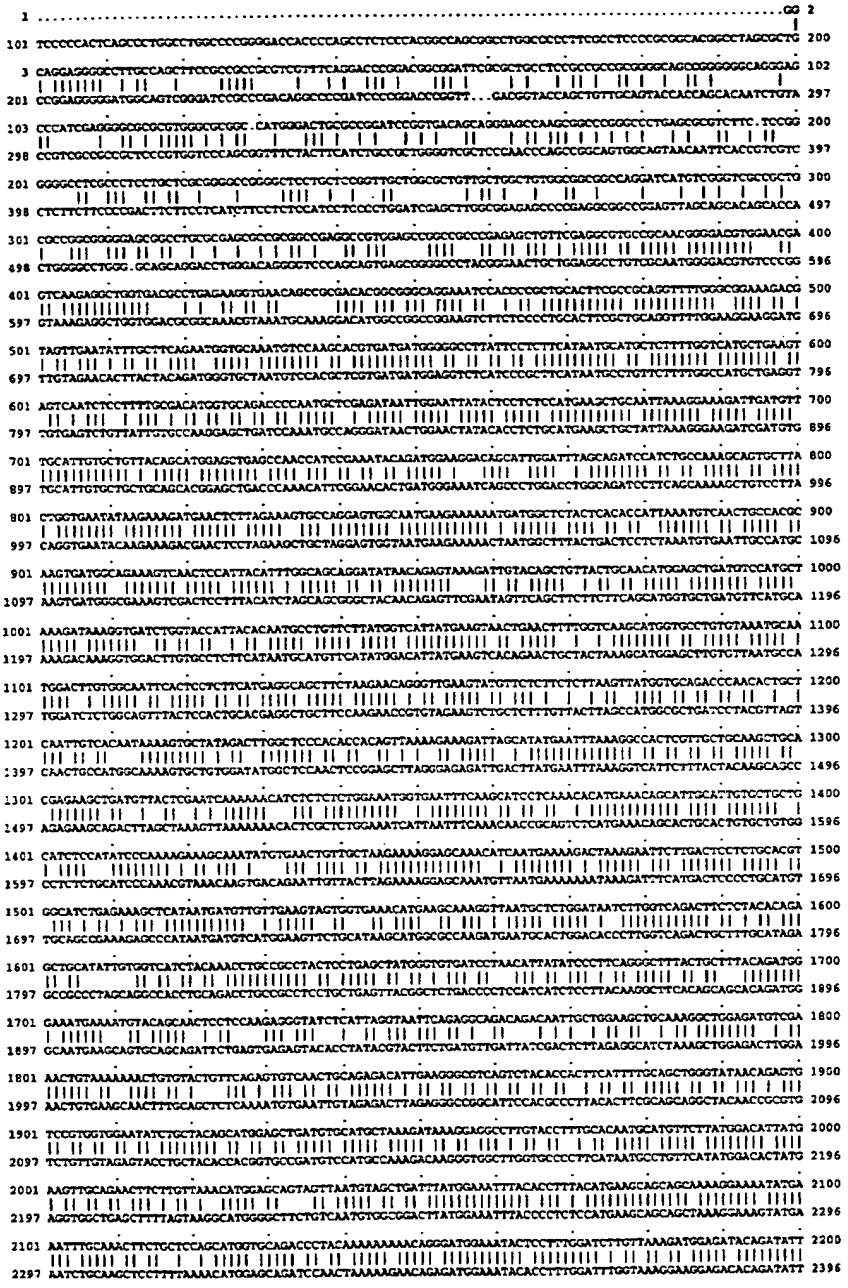

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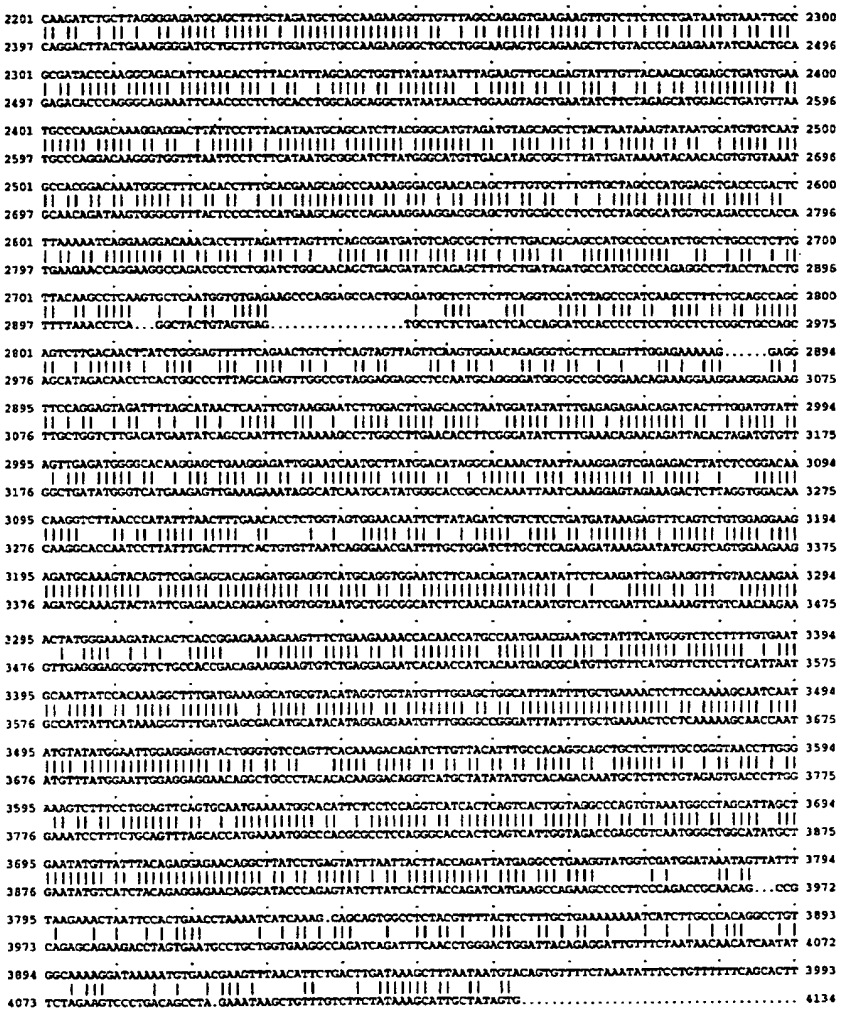

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3241 taatcaaagg agtagaaaga ctcttaggtg gacaacaagg caccaatcct tatttgactt
3301 ttcactgtgt taatcaggga acgattttgc tggatcttgc tccagaagat aaagaatatc
3361 agtcagtgga agaagagatg caaagtacta ttcgagaaca cagagatggt ggtaatgctg
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3421 gcggcatctt caacagatac aatgtcattc gaattcaaaa agttgtcaac aagaagttga
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3541 agcgcatgtt gtttcatggt tctcctttca ttaatgccat tattcataaa gggtttgatg
3601 agcgacatgc atacatagga ggaatgtttg gggccgggat ttattttgct gaaaactcct
3661 caaaaagcaa ccaatatgtt tatggaattg gaggaggaac aggctgccct acacacaagg
3721 acaggtcatg ctatatatgt cacagacaaa tgctcttctg tagagtgacc cttgggaaat
3781 cctttctgca gtttagcacc atgaaaatgg cccacgcgcc tccagggcac cactcagtca
3841 ttggtagacc gagcgtcaat gggctggcat atgctgaata tgtcatctac agaggagaac
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3961 ccgcaacagc cgcagagcag aagacctagt gaatgcctgc tggtgaaggc cagatcagat
4021 ttcaaccctgg gactggatta cagaggattg tttctaataa caacatcaat attctagaag
4081 tccctgacag cctagaaata agctgtttgt cttctataaa gcattgctat agtg
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SEQ. ID NO:8 (Tankyrase I protein sequence, GenBank Accession No. AF082556)

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MAASRRSQHHHHHHQQQLQPAPGASAPPPPPPPPLSPGLAPGTT
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PVVPAVSTSSAAGVAPNPAGSGSNNSPSSSSSPTSSSSSSPSSPGSSLAESPEAAGVS
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PLHFAAGFGRKDVVEHLLQMGANVHARDDGGLIPLHNACSFGHAEVVSLLLCQGADPN
ARDNWNYTPLHEAAIKGKIDVCIVLLQHGADPNIRNTDGKSALDLADPSAKAVLTGEY
KKDELLEAARSGNEEKLMALLTPLNVNCHASDGRKSTPLHLAAGYNRVRIVQLLLQHG
ADVHAKDKGGLVPLHNACSYGHYEVTELLLKHGACVNAMDLWQFTPLHEAASKNRVEV
CSLLLSHGADPTLVNCHGKSAVDMAPTPELRERLTYEFKGHSLLQAAREADLAKVKKT
LALEIINFKQPQSHETALHCAVASLHPKRKQVTELLLRKGANVNEKNKDFMTPLHVAA
ERAHNDVMEVLHKHGAKMNALDTLGQTALHRAALAGHLQTCRLLLSYGSDPSIISLQG
FTAAQMGNEAVQQILSESTPIRTSDVDYRLLEASKAGDLETVKQLCSSQNVNCRDLEG
RHSTPLHFAAGYNRVSVVEYLLHHGADVHAKDKGGLVPLHNACSYGHYEVAELLVRHG
ASVNVADLWKFTPLHEAAAKGKYEICKLLLKHGADPTKKNRDGNTPLDLVKEGDTDIQ
DLLKGDAALLDAAKKGCLARVQKLCTPENINCRDTQGRNSTPLHLAAGYNNLEVAEYL
LEHGADVNAQDKGGLIPLHNAASYGHVDIAALLIKYNTCVNATDKWAPTPLHEAAQKG
RTQLCALLLAHGADPTMKNQEGQTPLDLATADDIRALLIDAMPPEALPTCFKPQATVV
SASLISPASTPSCLSAASSIDNLTGPLAELAVGGASNAGDGAAGTERKEGEVAGLDMN
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VIRIQKVVNKKLRERFCHRQKEVSEENHNHHNERMLFHGSPFINAIIHKGFDERHAYI
GGMFGAGIYFAENSSKSNQYVYGIGGGTGCPTHKDRSCYICHRQMLFCRVTLGKSFLQ
FSTMKMAHAPPGHHSVIGRPSVNGLAYAEYVIYRGEQAYPEYLITYQIMKPEAPSQTA
TAAEQKT
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SEQ. ID NO:9 (ESP, GenBank Accession No. AA244138)

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  1 tcgacagaca attgctggaa gctgcaaagg ctggagatgt cgaaactgta aaaaaactgt
 61 gtactgttca gagtgtcaac tgcagagaca ttgaagggcg tcagtctaca ccacttcatt
121 ttgcagctgg gtataacaga gtgtccgtgg tggaatatct gctacagcat ggagctgatg
181 tgcatgctaa agataaagga ggccttgtac ctttgcacaa tgcatgttct tatggacatt
241 atgaagttgc agaacttctt gttaaacatg gagcagtagt taatgtagct gatttatgga
301 aatttacacc tttacatgaa gcagcagcaa aaggaaaata tgaaatttgc aaacttctgc
361 tccagcatgg tgcagaccct acaa
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SEQ. ID NO:10 (ESP, GenBank Accession No. AW157349)

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  1 ttttttaac tgtggtgtgg gagccaagtc tatagcactt ttattgtgac aattgagcag
 61 tgttgggtct gcaccataac ttaagagaag agaacatact tcaaccctgt tcttagaagc
121 tgcctcatga agaggagtga attgccacaa gtccattgca tttacacagg caccatgctt
181 gaccaaaagt tcagttactt cataatgacc ataagaacag gcattgtgta atggtaccag
241 atcacctta tctttagcat ggacatcagc tccatgttgc agtaacagct gtacaatctt
301 tactctgtta tatcctgctg ccaaatgtaa tggagttgac tttctgccat cacttgcgtg
361 gcagttgaca tttaatggtg tgagtagagc catcattttt tcttcattgc cactcctggc
421 actttctaag agttcatctt tcttatattc accagtaagc actgctttgg cagatggatc
481 tgctaaatcc aatgctgtcc ttccatctgt atttcggatg
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09558

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/68; G01N 33/53; C07H 21/04; A61K 39/40
US CL : 435/6, 7.1; 536/23.2; 424/139.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1; 536/23.2; 424/139.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, APS, MEDLINE, BIOSIS, DISSERTATION ABSTRACTS, EMBASE, CA SEARCH, INPADOC, search terms: tankyrase, telomere

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SMITH et al., Tankyrase, a Poly(ADP-Ribose) Polymerase at Human Telomeres, Science, 20 November 1998, Vol. 282 (5393), pages 1484-1487, especially page 1485, Fig. 2 and Abstract.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 AUGUST 2000	18 SEP 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HANKYEL T. PARK, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★